REMARKS

Claims 19-33 and 35-42 currently appear in this application. The Office Action of June 15, 2004, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicants respectfully request favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

Allowed Claims

Claims 19-33 are allowed.

Rejections under 35 U.S.C. 112

Claims 34-39 are rejected under 35 U.S.C. 112, first paragraph, because the application, while being enabling for detection of probes which comprise the pH or potential sensitive fluorophore attached to a steroid, to a head group of a sphingolipid or the head group of a lipid having two 14 carbon chains, which probes interact with a lipid layer, is said not to reasonably provide enablement for any pH or potential-sensitive fluorophore linked to a charged polymer without other structural information which interacts with any surface whatsoever.

This rejection is respectfully traversed. The present invention is directed to using a fluorophore-

incorporated surface that exhibits a change in an observable property that is pH-or potential-sensitive. Thus, if a test species is brought into contact with the fluorophore-incorporated surface, any observable change in the fluorophore will indicate binding of the test species to the surface. New claim 40 is submitted in order to more clearly define the invention. While pH- or potential-dependent fluorophores are well known, as are methods for binding these and other fluorophores to surfaces, such fluorophore-incorporated surfaces have never been used for the purposes of the present invention, that is, to indicate binding of a test species to a surface.

Of particular interest is an internet site describing molecular probes, www.probes.com. Specifically, http://www.probes.com.handbook/sections/2004.html, chapter 20, describes the state of the art concerning fluorophore-incorporated (conjugate) surfaces, including pH indicator-dexttrane conjugates, lipophilic pH indicators and other. This site also provides related links relevant to pH indicator conjugates, as well as other links helpful in understanding the field of molecular probes.

Thus, it is respectfully submitted that one skilled in the art, reading claim 40, would be able to used any pH- or

potential-sensitive fluorophore linked to a surface without undue experimentation, given the great number of pH- and potential-sensitive fluorophores available.

Claims 34, 35, and 37-39 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. The Examiner notes that claims 34, 35 and 37-39 encompass a genus of fluorophores whose fluorescence is dependent upon the binding or dissociation of a species at a surface, but only three specific species of such fluorophores are even suggested by the specification.

This rejection is respectfully traversed. Attention is directed to sections of chapter 20, namely, sections 20.1 and 20.4, from *Molecular Probes* cited above, copies of which are submitted herewith, which provide detailed information regarding pH- or potential-sensitive fluorophores that can be used for detection. It is clear from this printout that there are a great many pH- and potential-sensitive fluorophores that one skilled in the art can use in practicing the present invention. Because these fluorophores are well known to those

skilled in the art, it is respectfully submitted that one skilled in the art, knowing that a stable pH- or potential-sensitive fluorophore is required, can readily select from among the many such fluorophores available, without undue experimentation, to select one or more suitable for use in the detection method of the present invention.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By: _____
Anne M. Kornbau
Registration No. 25,884

AMK:srd
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
G:\BN\C\cohn\Barenholz1\Pto\AMD 15 NOV 04.doc

Search Results for: Chapter 20

Product Name Matches No Matches

Product Directory Matches No Matches

Handbook Matches 1 - 5 of 96 | Next 5 · All

Chapter 1 - Fluorophores and Their Amine-Reactive Derivatives
Chapter 1 — Fluorophores and Their Amine-Reactive Derivatives Section 1.1 — Introduction to Amine Modification Print * Common Applications for Amine-Reactive Probes * Labeling Biopolymers * Preparing the Optimal Bioconjugate * Derivatizing Low Molecular Weight Molecules * Reactivity of Amino Groups ...

Chapter 2 - Thiol-Reactive Probes
Chapter 2 — Thiol-Reactive Probes Section 2.1 — Introduction to Thiol Modification and Detection Print * Common Applications for Thiol-Reactive Probes * Labeling Biopolymers * Derivatizing Low Molecular Weight Molecules * Quantitating Thiols * Reactivity of Thiol Groups * Iodoacetamides * Maleimides ...

Chapter 10 - Enzyme Substrates
Chapter 10 — Enzyme Substrates Section 10.1 — Introduction to Enzyme Substrates and Their Reference Standards Print * Substrates Yielding Soluble Fluorescent Products * Substrates Derived from Water-Soluble Coumarins * Substrates Derived from Water-Soluble Green to Yellow Fluorophores * Substrates ...

Chapter 19 - Indicators for Ca2+, Mg2+, Zn2+ and Other Metal Ions
Chapter 19 — Indicators for Ca 2+, Mg 2+, Zn 2+ and Other Metal Ions Section 19.1 — Introduction to Ca{2+} Measurements with Fluorescent Indicators Print * Selection Criteria for Fluorescent Ca 2+ Indicators Section 19.2 — Fluorescent Ca {2+} Indicators Excited with UV Light Print * Fura-2, Indo-1 and ...

Chapter 20 - pH Indicators
Chapter 20 — pH Indicators Section 20.1 — Overview of pH Indicators Print Section 20.2 — Probes Useful at Near-Neutral pH Print * Fluorescein and Fluorescein Derivatives * Fluorescein and Its Diacetate * Carboxyfluorescein and Its Cell-Permeant Esters * BCECF and Its AM Ester * Fluorescein Sulfonic ...

Next 5 Matches... | All Matches...

Catalog Matches 1 - 5 of 5

Catalog of Cell Biology Products - Fluorescent and Biotinylated Microspheres for Tracing Applications
... highlights our 0.04 µm, 1 µm, and 10–15 µm beads. For a complete listing of

the Molecular Probes line of fluorescent microspheres, please refer to Chapter 6 in our Handbook of Fluorescent Probes and Research Products. FluoSpheres Microspheres for Tracking Cells Molecular Probes offers small (0 ...

Catalog of Cell Biology Products - Fluorescent and Biotinylated Dextrans for Tracing Applications

... on Molecular Probes fluorescent and biotinylated dextran conjugates — more than 50 products in various molecular weight ranges. Please see Chapter 14 in our Handbook of Fluorescent Probes and Research Products. Image Lineage tracing of three dorsal blastomeres of 32-cell Xenopus laevis embryos ...

Genomics and Molecular Biology Products - Nucleic Acid Dyes for Every Application

... For a wealth of detailed information about the structures, chemical properties and special applications for our nucleic acid stains, consult Chapter 8 of the Handbook of Fluorescent Probes and Research Products. Access the Handbook at /handbook. Image Handbook of Fluorescent Probes and Research ...

Genomics and Molecular Biology Products - Label Your Own Oligos

... . See the Handbook for More Information For information on alternative chemical labeling techniques for special applications, consult Chapter 8 of the Handbook of Fluorescent Probes and Research Products. Access the Handbook online at /handbook. Image Handbook of Fluorescent Probes ...

Catalog of Cell Biology Products - Plasma Membrane

... B) nile red bound to protein and phospholipid vesicles in BSA or DOPC. For more information on our complete line of lipid probes, please see Chapter 13 in our Handbook of Fluorescent Probes and Research Products. Previous Page Next Page

Additional Site Matches 1 - 5 of 33 | Next 5 · All

SYPRO Ruby Protein Blot Stain (PDF, 270KB)

... that emit at 450, 473, 488 or 532 nm. For information on appropriate filter sets to use with common laser scanners, see our reference table in Chapter 9.3 of our online Handbook of Fluorescent Probes and Research Products (www.probes.com/handbook). Subsequent Microanalysis Proteins may be analyzed by ...

BioProbes 25 - Reactive Dyes and Their Bioconjugates

... molecular weight molecules. The chemical and spectral properties, as well as the applications of these fluorophores, are extensively described in Chapter 1, Chapter 2 and Chapter 3 of our new Handbook of Fluorescent Probes and Research Chemicals, Seventh Edition. Ultraviolet Light–Excitable Dyes and ...

Aminoallyl dUTP (PDF, 123KB)

... the most stringent hybridization procedures. A complete description of succinimidyl ester reagents available from Molecular Probes can be found in Chapter 1 of the Hand book of Fluorescent Dyes and Research Products, avail able online at www.probes.com/handbook. The following protocol was optimized ...

BioProbes 25 - Phagocytosis and Receptor-Mediated Endocytosis

... (LDL), lactoferrin, transferrin and epidermal growth factor (EGF) Many more probes for investigating phagocytosis and endocytosis can be found in Chapter 16

and Chapter 19 of our Handbook. OxyBURST Reagents and Related Probes Fc OxyBURST Assay Reagents When soluble or surface-bound IgG immune complexes ...

aha-dUTP (PDF, 117KB)
... the most stringent hybridization procedures. A complete description of succinimidyl ester reagents available from Molecular Probes can be found in Chapter 1 of the Handbook of Fluorescent Dyes and Research Products, available online at www.probes.com/handbook. The following protocol was optimized for ...

Next 5 Matches... | **All Matches...**

Standard Search | Advanced Search

Chapter 20

View standard search instructions.

Search Reset the form.

---

invitrogen™

Section 20.1 — Overview of pH Indicators

The ability of dyes — notably litmus, phenolphthalein and phenol red — to change their color in response to a pH change has found widespread application in research and industry. However, fluorescent dyes provide the increased sensitivity required for optical pH measurements *inside* live cells. They also offer much greater spatial sampling capability when compared with microelectrode techniques. These advantages have spurred the development of improved fluorescent dyes that can sense pH changes within physiological ranges. Of course, many of the same fluorescent pH indicators can also be used as pH sensors in cell-free media.

To quantitatively measure pH, it is essential to match the indicator's pK_a to the pH of the experimental system. Consequently, the following two sections of this chapter are divided into pH indicators for use in environments with near-neutral pH (Section 20.2) and pH indicators for use in relatively acidic environments (Section 20.3). Intracellular pH is generally between ~6.8 and 7.4 in the cytosol and ~4.5 and 6.0 in the cell's acidic organelles. Unlike intracellular free Ca^{2+} concentrations, which can rapidly change by perhaps 100-fold, the pH inside a cell varies by only fractions of a pH unit, and such changes may be quite slow. Although the optical change of even the best fluorescent pH probes is usually relatively small, they have proven to be effective tools for investigating the role of intracellular pH in diverse physiological and pathological processes, including cell proliferation,[1] apoptosis,[2–5] muscle contraction,[6–8] malignancy,[9,10] multidrug resistance,[11–13] ion transport and homeostasis,[14–16] endocytosis [17] and Alzheimer's disease.[18,19]

Molecular Probes offers a variety of fluorescent pH indicators, their conjugates and other reagents for pH measurements in biological systems. Among these are several probes with unique optical response and specialized localization characteristics:

- Our visible light–excitable SNARF pH indicators enable researchers to determine intracellular pH in the physiological range using dual-emission or dual-excitation ratiometric techniques (Section 20.2), thus providing important tools for confocal laser-scanning microscopy and flow cytometry.
- Our LysoSensor probes, as well as indicators based on the Oregon Green fluorophore, can be used to estimate the pH in a cell's acidic organelles (Section 20.3).
- We also offer a number of fluorescent pH indicators coupled to dextrans (Section 20.4). Following loading into cells, indicator dextrans are extremely well retained, do not bind to cellular proteins and have a reduced tendency to compartmentalize.[20]

Families of pH indicators available from Molecular Probes are listed in Table 20.1 in approximate order of decreasing pK_a values.

1. J Cell Physiol 177, 109 (1998); **2.** J Biol Chem 271, 16260 (1996); **3.** J Biol Chem 270, 6235 (1995); **4.** Proc Natl Acad Sci U S A 93, 654 (1996); **5.** J Immunol Methods 221, 43 (1998); **6.** Pflugers Arch 435, 575 (1998); **7.** Am J Physiol 275, H1788 (1998); **8.** J Physiol 512, 831 (1998); **9.** Cancer Res 54, 5670 (1994); **10.** Nat Med 3, 177 (1997); **11.** Biochemistry 35, 2811 (1996); **12.** Biophys J 69, 883 (1995); **13.** Proc Natl Acad Sci U S A 91, 1128 (1994); **14.** J Neurochem 71, 1051 (1998); **15.** Biochemistry 35, 13419 (1996); **16.** J Physiol 506, 415 (1998); **17.** Proc Natl Acad Sci U S A 92, 3156 (1995); **18.** Biochem Biophys Res Commun 194, 537 (1993); **19.** Neuroreport 9, 1553 (1998); **20.** Methods Cell Biol 29, 59 (1989).

* * * * *

Table 20.1 — Molecular Probes' pH indicator families, in order of decreasing pK_a

Parent Fluorophore	pH Range	Typical Measurement
SNARF indicators	6.0–8.0	Emission ratio 580/640 nm
HPTS (pyranine)	7.0–8.0	Excitation ratio 450/405 nm
BCECF	6.5–7.5	Excitation ratio 490/440 nm
Fluoresceins and carboxyfluoresceins	6.0–7.2	Excitation ratio 490/450 nm
LysoSensor Green DND-189	4.5–6.0	Single emission 520 nm
Oregon Green dyes	4.2–5.7	Excitation ratio 510/450 nm or excitation ratio 490/440 nm
LysoSensor Yellow/Blue DND-160	3.5–6.0	Emission ratio 450/510 nm

* * * * *

Section 20.2 — Probes Useful at Near-Neutral pH

Fluorescein and Fluorescein Derivatives

Fluorescein and many of its derivatives exhibit multiple, pH-dependent ionic equilibria.[1–5] Both the phenol and carboxylic acid functional groups of fluorescein are almost totally ionized in aqueous solutions above pH 9 (Figure 20.1). Acidification of the fluorescein dianion first protonates the phenol (pK_a ~6.4) to yield the fluorescein monoanion, then the carboxylic acid (pK_a <5) to produce the neutral species of fluorescein. Further acidification generates a fluorescein cation (pK_a ~2.1). Only the monoanion and dianion of fluorescein are fluorescent, with quantum yields of 0.37 and 0.93, respectively. However, excitation of either the neutral or cationic species is reported to produce emission from the anion with effective quantum yields of 0.31 and 0.18, respectively.[2] A further equilibrium involves formation of a colorless, nonfluorescent lactone (Figure 20.1). The lactone is not formed in aqueous solution above pH 5 but may be the dominant form of neutral fluorescein in solvents such as acetone. The pH-dependent absorption spectra of fluorescein (Figure 20.2) clearly show the blue shift and decreased absorptivity indicative of the formation of protonated species. However, the fluorescence emission spectrum of most fluorescein derivatives, even in acidic solution, is dominated by the dianion, with only small contributions from the monoanion. Consequently, the wavelength and shape of the emission spectra resulting from excitation close to the dianion absorption peak at 490 nm are relatively independent of pH, but the fluorescence intensity is dramatically reduced at acidic pH (Figure 20.2).

Molecular Probes offers a broad variety of fluorescein-derived reagents and fluoresceinated probes that can serve as sensitive fluorescent pH indicators in a wide range of applications. Chemical substitutions of fluorescein may shift absorption and fluorescence maxima and change the pK_a of the dye; however, the effects of acidification on the spectral characteristics illustrated in Figure 20.2 are generally maintained in all fluorescein derivatives.

Fluorescein and Its Diacetate

The cell-permeant fluorescein diacetate (FDA, F1303) is still occasionally used to measure intracellular pH,[6] as well as to study cell adhesion[7] or, in combination with propidium iodide (P1304MP, P3566, P21493; Section 8.1), to determine cell viability.[8,9] However, fluorescein (F1300), which is formed by intracellular hydrolysis of FDA, rapidly leaks from cells (Figure 15.3). Thus, other cell-permeant dyes such as the acetoxymethyl (AM) esters of BCECF and calcein are now preferred for intracellular pH measurements and cell viability assays (Section 15.2).

Carboxyfluorescein and Its Cell-Permeant Esters

The high leakage rate of fluorescein from cells makes it very difficult to quantitate intracellular pH because the decrease in the cell's fluorescence due to dye leakage cannot be easily distinguished from that due to acidification. The use of carboxyfluorescein diacetate (CFDA, C195) for intracellular pH measurements partially addresses this problem.[10] CFDA is moderately permeant to most cell membranes and, upon hydrolysis by intracellular nonspecific esterases, forms carboxyfluorescein (5(6)-FAM, C194; FluoroPure Grade, C1904), which has a pH-dependent spectral response very similar to that of fluorescein. As compared to fluorescein, carboxyfluorescein contains an extra negative charge and is therefore better retained in cells[11]

(Figure 15.3). The mixed-isomer preparation of CFDA (C195) is usually adequate for intracellular pH measurements because the single isomers of carboxyfluorescein exhibit essentially identical pH-dependent spectra with a pK_a ~6.5. For experiments requiring a pure isomer, the single-isomer preparations of carboxyfluorescein (C1359, C1360; Section 1.5) and CFDA (C1361, C1362; Section 15.2) are available. In addition, we offer the AM ester of CFDA (5-CFDA, AM, C1354; Figure 15.8), which is electrically neutral and facilitates cell loading. Upon hydrolysis by intracellular esterases, this AM ester also yields carboxyfluorescein.[12-14]

Intracellular pH measurements have been made using carboxyfluorescein,[15-17] although the spectral and pK_a properties of the SNARF and BCECF dyes (see below) make these indicators superior probes for most pH studies. Carboxyfluorescein is also commonly employed as a polar tracer (Section 14.3), and CFDA and its AM ester are used for monitoring cell viability,[14,18] apoptosis [19-22] and cell adhesion [11,23,24] (Section 15.2, Section 15.5 and Section 15.6).

BCECF and Its AM Ester

Although carboxyfluorescein is better retained in cells than is fluorescein, its pK_a of ~6.5 is lower than the cytosolic pH of most cells (pH ~6.8–7.4). Consequently, its fluorescence change is less than optimal for detecting small pH changes above pH 7. Since its introduction by Roger Tsien in 1982,[25,26] the polar fluorescein derivative BCECF (B1151) and its membrane-permeant AM ester (B1150, B1170, B3051) have become the most widely used fluorescent indicators for estimating intracellular pH. Also, a flow cytometric assay has been developed that uses BCECF to estimate the concentration of intracellular K^+.[27] BCECF's four to five negative charges at pH 7–8 improve its retention in cells (Figure 15.3), and its pK_a of 6.98 is ideal for typical intracellular pH measurements.

As with fluorescein and carboxyfluorescein, absorption of the phenolate anion (basic) form of BCECF is red-shifted and has increased molar absorptivity relative to the protonated (acidic) form (Figure 20.3); there is little, if any, pH-dependent shift in the fluorescence emission spectrum of BCECF upon excitation at 505 nm (Figure 20.3, Figure 20.4). BCECF is typically used as a dual-excitation ratiometric pH indicator. Signal errors caused by variations in concentration, path length, leakage and photobleaching are greatly reduced with ratiometric methods (see "Technical Focus: Loading and Calibration of Intracellular Ion Indicators" in Section 19.1). Intracellular pH measurements with BCECF are made by determining the pH-dependent ratio of emission intensity (detected at 535 nm) when the dye is excited at ~490 nm versus the emission intensity when excited at its isosbestic point of ~440 nm (Figure 20.3, Figure 20.4). Because BCECF's absorption at 440 nm is quite weak, increasing the denominator wavelength to ~450 nm provides improved signal-to-noise characteristics for ratio imaging applications.[28-30] As with other intracellular pH indicators, *in situ* calibration of BCECF's fluorescence response is usually accomplished using 10–50 µM nigericin (N1495, see below) in the presence of 100–150 mM K^+ to equilibrate internal and external pH.[10,31] Alternative calibration methods have also been reported.[32-34]

Loading of live cells for measurement of intracellular pH is readily accomplished by incubating cell suspensions or adherent cells in a 1–10 µM solution of the AM ester of BCECF. At least three different molecular species can be obtained in synthetic preparations of the AM ester of BCECF; however, all three forms shown in Figure 20.5 appear to be converted to the same product — BCECF acid (B1151, Figure 20.6) — by intracellular esterase hydrolysis. Although we can readily prepare the pure tri(acetoxymethyl) ester form (Form I in Figure 20.5), some researchers have found that cell loading with a mixture of the lactone Forms II and III is more efficient. Consequently, we produce BCECF AM predominantly as a mixture of Forms II and III with a typical percentage composition ratio of 45:55, as determined by HPLC, NMR and mass spectrometry. Because the molecular weights of the different species are not equal, the effective molecular weight for each production lot is reported on the product's label. The AM ester of BCECF is available in a single 1 mg vial (B1150), specially packaged as a set of 20 vials that

each contains 50 µg (B1170) and as a 1 mg/mL solution (~1.6 mM) in anhydrous dimethylsulfoxide (DMSO) (B3051). We highly recommend purchasing the set of 20 vials in order to reduce the potential for product deterioration caused by exposure to moisture.

Our bibliography for BCECF AM lists more than 1200 journal citations, including references to the use of BCECF AM to investigate:

- Cl^-/HCO_3^- exchange [35–37]
- K^+/H^+ exchange [38,39]
- Lactate transport and metabolism [40–42]
- Na^+/H^+ exchange [43–46]
- Na^+/Ca^{2+} exchange [47,48]
- NH_4^+ transport [49,50]
- Apoptosis [51–55] (Section 15.5)
- Cytotoxicity [56–59]
- Multidrug resistance [60–63] (Section 15.6)
- Cell volume changes [64–66]
- Cytosolic pH regulation in osteoblasts [67] and osteoclasts [68,69]
- Phagocytosis [70–72] (Section 16.1)

The cell-impermeant BCECF acid (B1151, Figure 20.6) is useful for pH measurements in intercellular spaces of epithelial cell monolayers,[73] interstitial spaces of normal and neoplastic tissue [74] and isolated cell fractions.[75] BCECF has also been employed for two-photon fluorescence lifetime imaging of the skin stratum corneum to detect aqueous acid pockets within the lipid-rich extracellular matrix.[76] The free acid of BCECF can be loaded into cells by microinjection [34] or electroporation or by using our Influx pinocytic cell-loading reagent (I14402, Section 19.8). It has also been loaded into bacterial cells by brief incubation at pH ~2.[77–79] In addition to the cell-permeant BCECF AM and cell-impermeant BCECF acid, Molecular Probes offers dextran conjugates of BCECF (D1878, D1880; Section 20.4).

Fluorescein Sulfonic Acid and Its Diacetate

The fluorescein-5-(and 6-)sulfonic acid (F1130) is much more polar than carboxyfluorescein. Consequently, once inside cells or liposomes, it is relatively well retained. Some cells can be loaded directly with 5-sulfofluorescein diacetate [80–83] (SFDA, S1129). Direct ratiometric measurement of the pH in the trans-Golgi of live human fibroblasts was achieved by simultaneously microinjecting liposomes loaded with both fluorescein sulfonic acid and sulforhodamine 101 [84] (S359, Section 14.3). Fluorescein-5-(and 6-)sulfonic acid is more commonly used to measure barrier permeability of membranes (Section 14.3).

Chemically Reactive Fluorescein Diacetates

One means for overcoming the cell leakage problem common to the above pH indicators, including BCECF, is to trap the indicator inside the cell via conjugation to intracellular constituents. The chloromethyl derivatives CellTracker Green CMFDA (C2925, C7025; Figure 14.11) and chloromethyl SNARF-1 (C6826, see below) probably have the greatest potential for relatively long-term cell tracing and pH studies. In many cell types, the weakly thiol-reactive chloromethyl moiety of CMFDA reacts with intracellular thiols, including glutathione and proteins, to yield well-retained products (Figure 15.3). Cleavage of the acetate groups of the CMFDA conjugate by intracellular esterases yields a conjugate that retains the pH-dependent spectral properties of fluorescein.

Similarly, the amine-reactive succinimidyl ester of CFDA (5(6)-CFDA SE, also called CFSE,

C1157) can potentially be used for long-term pH studies of live cells, producing a conjugate with the pH-sensitive properties of carboxyfluorescein. Because it is intrinsically more reactive, the succinimidyl ester of CFDA is more likely to react at sites on the extracellular surface than is CMFDA.

Carboxynaphthofluorescein

Carboxynaphthofluorescein (C652, Figure 20.7) has pH-dependent red fluorescence (excitation/emission maxima ~598/668 nm at pH >9) with a relatively high pK_a of ~7.6. The long-wavelength fluorescent pH-dependent spectra of carboxynaphthofluorescein have been exploited in the construction of fiber-optic pH sensors.[85,86] This long-wavelength pH indicator is also available in membrane-permeant diacetate form (C13196) for passive intracellular loading and as an amine-reactive succinimidyl ester (C653, Section 20.4) for preparing pH-sensitive conjugates.

SNARF and SNAFL pH Indicators

Our patented seminaphthorhodafluors (SNARF dyes) and seminaphthofluoresceins (SNAFL dyes) are visible light–excitable fluorescent pH indicators developed at Molecular Probes.[87,88] The SNARF and SNAFL indicators have both dual-emission and dual-excitation properties, making them particularly useful for confocal laser-scanning microscopy[89–92] (Figure 20.8), flow cytometry[30,93–95] and microplate reader–based measurements.[96] The dual-emission properties of SNARF dyes may make these dyes the preferred probes for use in fiber-optic pH sensors.[97–99] The fluorophores can be excited by the 488 or 514 nm spectral lines of the argon-ion laser and are sensitive to pH values within the physiological range. Dextran conjugates of the SNARF dyes are described in Section 20.4.

Carboxy SNARF-1 Dye and Its Cell-Permeant Ester

The carboxy SNARF-1 dye (C1270, Figure 20.9), which is easily loaded into cells as its cell-permeant AM ester acetate (C1271, C1272), has a pK_a of about 7.5 at room temperature and 7.3–7.4 at 37°C. Thus, carboxy SNARF-1 is useful for measuring pH changes between pH 7 and 8. Like fluorescein and BCECF, the absorption spectrum of the carboxy SNARF-1 pH indicator undergoes a shift to longer wavelengths upon deprotonation of its phenolic substituent (Figure 20.10). In contrast to the fluorescein-based indicators, however, carboxy SNARF-1 also exhibits a significant pH-dependent emission shift from yellow-orange to deep red fluorescence under acidic and basic conditions, respectively (Figure 20.11, Figure 20.12). This pH dependence allows the ratio of the fluorescence intensities from the dye at two emission wavelengths (Figure 20.8) — typically 580 nm and 640 nm — to be used for quantitative determinations of pH (see "Technical Focus: Loading and Calibration of Intracellular Ion Indicators" in Section 19.1). For practical purposes, it is often desirable to bias the detection of carboxy SNARF-1 fluorescence towards the less fluorescent acidic form by using an excitation wavelength between 488 nm and the excitation isosbestic point at ~530 nm, yielding balanced signals for the two emission-ratio components (Figure 20.11, Figure 20.13). When excited at 488 nm, carboxy SNARF-1 exhibits an emission isosbestic point of ~610 nm and a lower fluorescent signal than obtained with 514 nm excitation.[92] Alternatively, when excited by the 568 nm spectral line of the Ar–Kr laser found in some confocal laser-scanning microscopes, carboxy SNARF-1 exhibits a fluorescence increase at 640 nm as the pH increases and an emission isosbestic point at 585 nm.[92] As with other ion indicators, intracellular environments may cause significant changes to both the spectral properties and pK_a of carboxy SNARF-1,[100–103] and the indicator should always be calibrated in the system under study.

The spectra of carboxy SNARF-1 are well resolved from those of fura-2,[104,105] and indo-1 [106] (Section 19.2), as well as those of the fluo-3,[105,107,108], fluo-4, Calcium Green and Oregon Green

488 BAPTA Ca^{2+} indicators (Section 19.3), permitting simultaneous measurements of intracellular pH and Ca^{2+} (Figure 20.14). Carboxy SNARF-1 has also been used in combination with the Na^+ indicator SBFI (S1262, S1263, S1264; Section 21.1) to simultaneously detect pH and Na^+ changes in heart mitochondria.[109] The relatively long-wavelength excitation and emission characteristics of carboxy SNARF-1 facilitate studies in autofluorescent cells [110] and permit experiments that employ the anion-transport inhibitor DIDS [111,112] (D337, Section 16.3), amiloride derivatives [111,113] (Section 16.3), caged probes (Section 5.3) and other modifiers of cell function that require UV light excitation. In addition, the ability to excite carboxy SNARF-1 near 490 nm and to observe red fluorescence beyond 600 nm permits its use as a Ca^{2+}-*insensitive* reference dye in order to make ratiometric measurements of intracellular Ca^{2+} with the nonratiometric Ca^{2+} indicators fluo-3,[114–117] fluo-4 and Calcium Green-2.[118] Incubation of cells for several hours after loading with carboxy SNARF-1, AM ester, acetate, results in compartmentally selective retention of the dye allowing *in situ* measurements of mitochondrial pH [119] (Figure 20.15).

SNARF-4F and SNARF-5F Dyes

Although the carboxy SNARF-1 indicator possesses excellent spectral properties, its pK_a of ~7.5 may be too high for measurements of intracellular pH in some cells. For quantitative measurements of pH changes in the typical cytosolic range (pH ~6.8–7.4), we now recommend SNARF-5F carboxylic acid (Figure 20.16), which has a pK_a value of ~7.2, as the indicator with the best spectral properties for estimating cytosolic pH (Figure 20.17). SNARF-4F carboxylic acid (Figure 20.18) has a somewhat more acidic pH sensitivity maximum (pK_a ~6.4) but retains its dual emission spectral properties (Figure 20.19). Both SNARF-4F and SNARF-5F [120] allow dual-excitation and dual-emission ratiometric pH measurements, making them compatible with the same instrument configurations used for carboxy SNARF-1 in ratio imaging and flow cytometry applications. SNARF-4F and SNARF-5F are available as free carboxylic acids (S23920, S23922) and as cell-permeant diacetate derivatives (S23921, S23923).

Chloromethyl SNARF-1 Acetate

Our 5-(and 6-)chloromethyl SNARF-1 acetate (C6826) contains a chloromethyl group that is mildly reactive with intracellular thiols (Figure 14.20), forming adducts that should improve cellular retention of the SNARF fluorophore (Figure 14.22). As with CellTracker Green CMFDA (see above), improved retention of this conjugate in cells may permit monitoring of intracellular pH over longer time periods than is possible with other intracellular pH indicators. Like our CellTracker dyes (Section 14.2), it is also useful simply as a long-term cell tracer.

Carboxy SNAFL-1

In contrast to fluorescein and its derivatives, the acidic form of the SNAFL-1 pH indicator has higher fluorescence quantum yields than their basic forms.[121,122] SNAFL indicators can be used for either dual-emission [121] (Figure 20.20) or dual-excitation (Figure 20.21) ratiometric pH measurements. SNAFL indicators have also been incorporated in fluorescence lifetime–based pH and CO_2 sensors and in fiber-optic pH sensors.[77] Carboxy SNAFL-1 is available as a free acid (C1255) and as a cell-permeant diacetate derivative (C1256).

8-Hydroxypyrene-1,3,6-Trisulfonic Acid (HPTS)

8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS, also known as pyranine; H348; Figure 20.22) is an inexpensive, highly water-soluble, membrane-impermeant pH indicator with a pK_a of ~7.3 in aqueous buffers.[123] The pK_a of HPTS is reported to rise to 7.5–7.8 in the cytosol of some cells.[124] Unlike indicators based on the SNARF and fluorescein dyes, there is no membrane-permeant form of HPTS available. Consequently, HPTS must be introduced into cells by microinjection,

electroporation [125] or liposome-mediated delivery,[126-128] through ATP-gated ion channels,[129] or by other relatively invasive means (Table 14.1). HPTS exhibits a pH-dependent absorption shift (Figure 20.23), allowing ratiometric measurements using an excitation ratio of 450/405 nm.[130]

The unique pH-dependent spectral properties, high water solubility and low cost of HPTS make its applications numerous. They include:

- Detecting proton permeability in liposomes and cells [131-133]
- Investigating pH-mediated changes of intracellular Ca^{2+} [134]
- Fiber-optic sensing of oxygen and carbon dioxide,[135,136] ammonia [137] and enzymatic activity [138]
- Detecting bioenergetically linked proton-transfer processes [139-143]
- Measuring acidity of lysosomes and other organelles (Section 20.3)
- Detecting membrane fusion and lysis [144,145]
- Following endocytosis [146-148] (Section 16.1)
- Detecting targeted intracellular delivery of liposome-encapsulated molecules [126,127,149,150]

We also offer a dextran conjugate of HPTS (D7179, Section 20.4).

Auxiliary Probes for pH Measurements

In addition to the fluorescent pH indicators described above and in the subsequent sections, Molecular Probes provides nigericin, which is widely used for calibrating intracellular pH indicators, as well as some unique caged compounds that can be used for localized generation of protons by UV photolysis.

Nigericin

Intracellular calibration of the fluorescence response of cytosolic pH indicators is typically performed using the K^+/H^+ ionophore nigericin (N1495), which causes equilibration of intracellular and extracellular pH in the presence of a depolarizing concentration of extracellular K^+ [10,31] (see "Technical Focus: Loading and Calibration of Intracellular Ion Indicators" in Section 19.1). Nett and Deitmer have compared this technique with calibrations performed by direct insertion of pH-sensitive microelectrodes in leech giant glial cells.[34]

Caged Protons

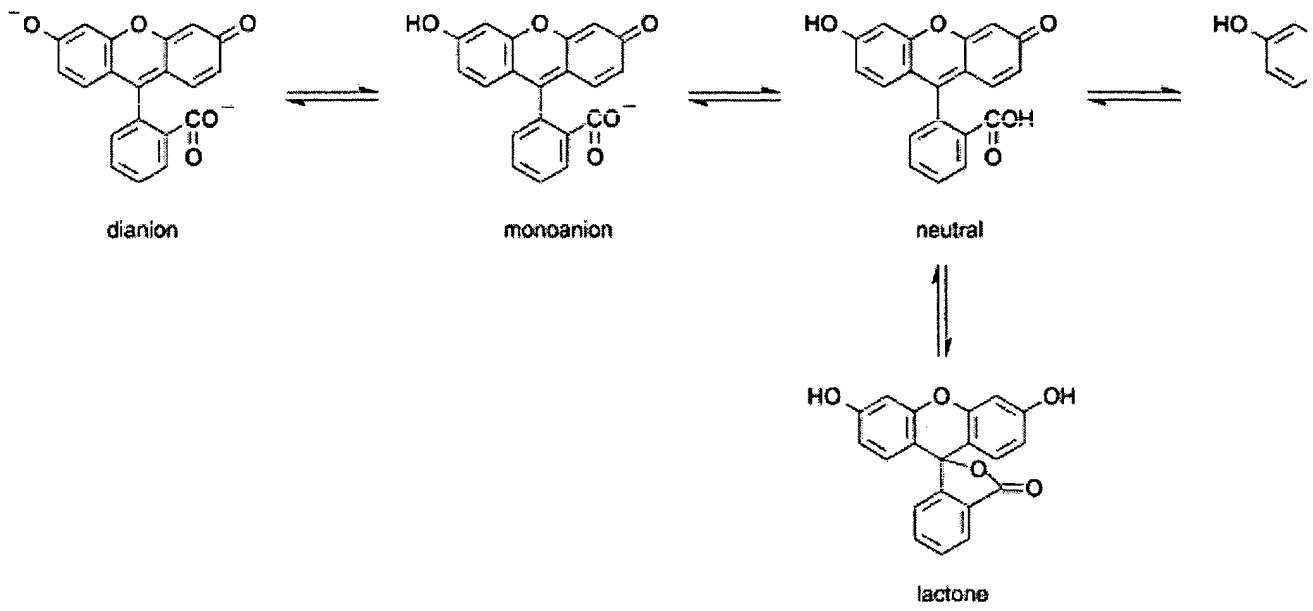
Photolysis of NPE-caged phosphate [151-154] (N7065) liberates inorganic phosphate, which rapidly ionizes to release a proton. This caged phosphate can be used to produce a pulse of phosphate and to generate a photolysis-dependent proton release that results in a rapid drop in pH. See Section 5.3 for a more complete discussion of the properties and applications of caged probes.

1. J Fluorescence 6, 147 (1996); **2.** Spectrochim Acta A 51, 7 (1995); **3.** Photochem Photobiol 60, 435 (1994); **4.** J Luminescence 10, 381 (1975); **5.** J Phys Chem 75, 245 (1971); **6.** FEBS Lett 341, 125 (1994); **7.** J Immunol Methods 157, 117 (1993); **8.** Methods Mol Biol 43, 211 (1995); **9.** J Histochem Cytochem 33, 77 (1985); **10.** Biochemistry 18, 2210 (1979); **11.** J Immunol Methods 172, 115 (1994); **12.** Biochemistry 34, 1606 (1995); **13.** Cytometry 13, 739 (1992); **14.** J Immunol Methods 130, 251 (1990); **15.** Appl Environ Microbiol 63, 178 (1997); **16.** Cytometry 19, 235 (1995); **17.** Photochem Photobiol 60, 274 (1994); **18.** Anticancer Res 14, 927 (1994); **19.** J Immunol Methods 243, 191 (2000); **20.** J Immunol Methods 243, 155 (2000); **21.** J Cell Biol 134, 757 (1996); **22.** Exp Cell Res 211, 322 (1994); **23.** Micron 31, 41 (2000); **24.** J Cell Biol 124, 609 (1994); **25.** Proc Natl Acad Sci U S A 81, 7436 (1984); **26.** J Cell Biol 95, 189 (1982); **27.** Cytometry 28, 42 (1997); **28.** Methods Cell Biol 30, 157 (1989);

29. Cell Biology: A Laboratory Handbook, 2nd Ed., Vol. 3, Celis JE, Ed. pp. 380–386 (1998); **30.** Methods Cell Biol 41, 135 (1994); **31.** Methods Enzymol 192, 38 (1990); **32.** J Cell Physiol 151, 596 (1992); **33.** J Fluorescence 2, 191 (1992); **34.** Biophys J 71, 394 (1996); **35.** Kidney Int 53, 432 (1998); **36.** Am J Physiol 274, F358 (1998); **37.** J Membr Biol 159, 253 (1997); **38.** Biochemistry 34, 15157 (1995); **39.** J Biol Chem 272, 25668 (1997); **40.** J Biol Chem 273, 15920 (1998); **41.** J Biol Chem 273, 27162 (1998); **42.** J Biol Chem 271, 861 (1996); **43.** J Biol Chem 273, 20828 (1998); **44.** J Cell Biol 140, 335 (1998); **45.** J Biol Chem 273, 2035 (1998); **46.** J Biol Chem 273, 8790 (1998); **47.** J Neurochem 71, 1051 (1998); **48.** J Biol Chem 270, 9137 (1995); **49.** Am J Physiol 273, F817 (1997); **50.** Plant Physiol 113, 451 (1997); **51.** J Biol Chem 276, 514 (2001); **52.** J Mol Cell Cardiol 30, 519 (1998); **53.** J Biol Chem 273, 12662 (1998); **54.** J Biol Chem 270, 6235 (1995); **55.** J Biol Chem 271, 16260 (1996); **56.** Proc Natl Acad Sci U S A 98, 3434 (2001); **57.** Biotechniques 23, 139 (1997); **58.** J Microbiol Methods 28, 35 (1997); **59.** J Immunol Methods 172, 255 (1994); **60.** Eur J Biochem 243, 219 (1997); **61.** Am J Physiol 274, C182 (1998); **62.** Br J Cancer 75, 810 (1997); **63.** Biochemistry 36, 11169 (1997); **64.** Meth Neurosci 27, 361 (1995); **65.** Pflugers Arch 435, 74 (1997); **66.** Neuroscience 69, 283 (1995); **67.** Miner Electrolyte Metab 20, 16 (1994); **68.** Am J Physiol Cell Physiol 279, C751 (2000); **69.** J Biol Chem 272, 6354 (1997); **70.** J Biol Chem 272, 29810 (1997); **71.** J Leukoc Biol 62, 329 (1997); **72.** J Biol Chem 271, 2005 (1996); **73.** J Membr Biol 140, 89 (1994); **74.** Cancer Res 54, 5670 (1994); **75.** Biochemistry 35, 13419 (1996); **76.** Biophys J 83, 1682 (2002); **77.** Anal Chem 71, 154 (1999); **78.** Mol Membr Biol 13, 173 (1996); **79.** Biochim Biophys Acta 1115, 75 (1991); **80.** J Cell Biol 111, 3129 (1990); **81.** J Immunol Methods 133, 87 (1990); **82.** FEBS Lett 200, 203 (1986); **83.** Biotechniques 3, 270 (1985); **84.** J Biol Chem 270, 4967 (1995); **85.** Mikrochim Acta 108, 133 (1992); **86.** Anal Chem 69, 863 (1997); **87.** Anal Biochem 194, 330 (1991); **88.** US 4,945,171; **89.** Methods Enzymol 302, 341 (1999); **90.** Micron 24, 573 (1993); **91.** Am J Physiol 275, H1937 (1998); **92.** Biophys J 66, 942 (1994); **93.** Cytometry 14, 916 (1993); **94.** J Immunol Methods 221, 43 (1998); **95.** J Cell Physiol 177, 109 (1998); **96.** Am J Physiol 273, C1783 (1997); **97.** J Biomed Mater Res 39, 9 (1998); **98.** J Immunol Methods 159, 145 (1993); **99.** Anal Chem 65, 2329 (1993); **100.** J Photochem Photobiol B 37, 18 (1997); **101.** Pflugers Arch 427, 332 (1994); **102.** Anal Biochem 204, 65 (1992); **103.** J Fluorescence 2, 75 (1992); **104.** J Cell Physiol 161, 129 (1994); **105.** Cell Calcium 19, 337 (1996); **106.** Endocrinology 133, 972 (1993); **107.** J Physiol 528 Pt 1, 25 (2000); **108.** Cytometry 24, 99 (1996); **109.** J Biol Chem 270, 672 (1995); **110.** Am J Physiol 267, L211 (1994); **111.** J Biol Chem 270, 1315 (1995); **112.** Pflugers Arch 417, 234 (1990); **113.** Arch Biochem Biophys 356, 25 (1998); **114.** J Biol Chem 270, 29781 (1995); **115.** J Biol Chem 269, 30636 (1994); **116.** Biochem J 289, 373 (1993); **117.** Cytometry 11, 923 (1990); **118.** Pflugers Arch 430, 579 (1995); **119.** Biotechniques 30, 804 (2001); **120.** Bioorg Med Chem Lett 11, 2903 (2001); **121.** J Photochem Photobiol B 28, 19 (1995); **122.** Anal Chim Acta 274, 47 (1993); **123.** Fresenius Z Anal Chem 314, 119 (1983); **124.** Anal Biochem 167, 362 (1987); **125.** J Bacteriol 177, 1017 (1995); **126.** Pharm Res 14, 1203 (1997); **127.** Proc Natl Acad Sci U S A 94, 8795 (1997); **128.** Curr Eye Res 16, 1073 (1997); **129.** Am J Physiol 275, C1158 (1998); **130.** Proc Natl Acad Sci U S A 92, 3156 (1995); **131.** Biophys J 70, 339 (1996); **132.** Biophys J 71, 3091 (1996); **133.** Biophys J 68, 1518 (1995); **134.** J Physiol 530, 405 (2001); **135.** Anal Chem 67, 2264 (1995); **136.** Analyst 121, 339 (1996); **137.** Anal Chim Acta 185, 321 (1986); **138.** Anal Biochem 252, 190 (1997); **139.** J Biol Chem 276, 25480 (2001); **140.** Biochemistry 37, 2496 (1998); **141.** Biochemistry 34, 8820 (1995); **142.** Biophys J 71, 1011 (1996); **143.** Biophys J 73, 2638 (1997); **144.** Biochemistry 36, 6251 (1997); **145.** J Cell Biol 131, 679 (1995); **146.** J Cell Biol 121, 305 (1993); **147.** Biochemistry 37, 12875 (1998); **148.** J Biochem (Tokyo) 117, 34 (1995); **149.** Biochemistry 36, 66 (1997); **150.** J Biol Chem 271, 7249 (1996); **151.** FEBS Lett 405, 81 (1997); **152.** J Physiol 451, 247 (1992); **153.** Biophys J 72, 1780 (1997); **154.** J Mol Biol 184, 645 (1985).

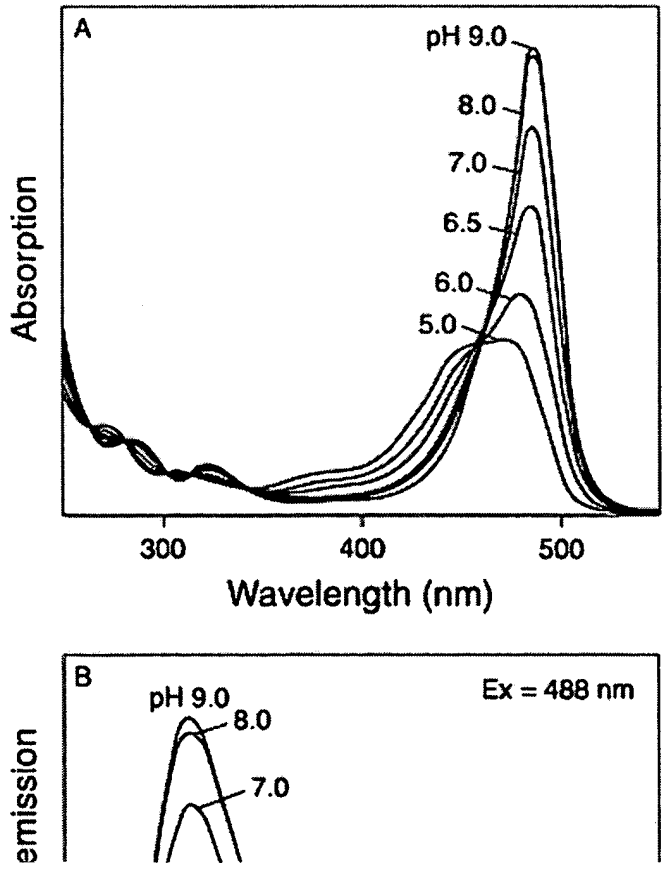
* * * * *

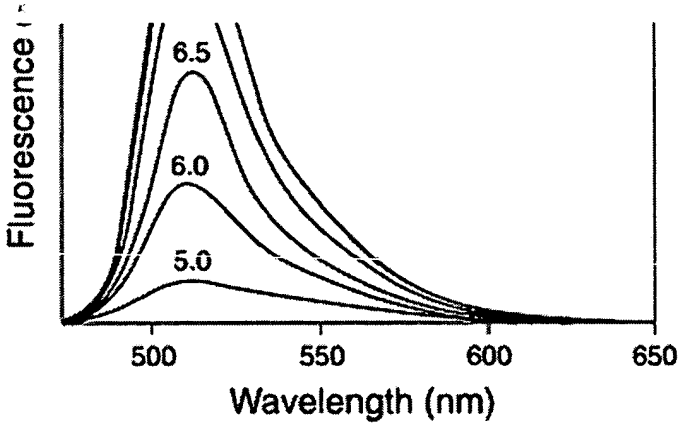
Figure 20.1 Ionization equilibria of fluorescein.



* * * * *

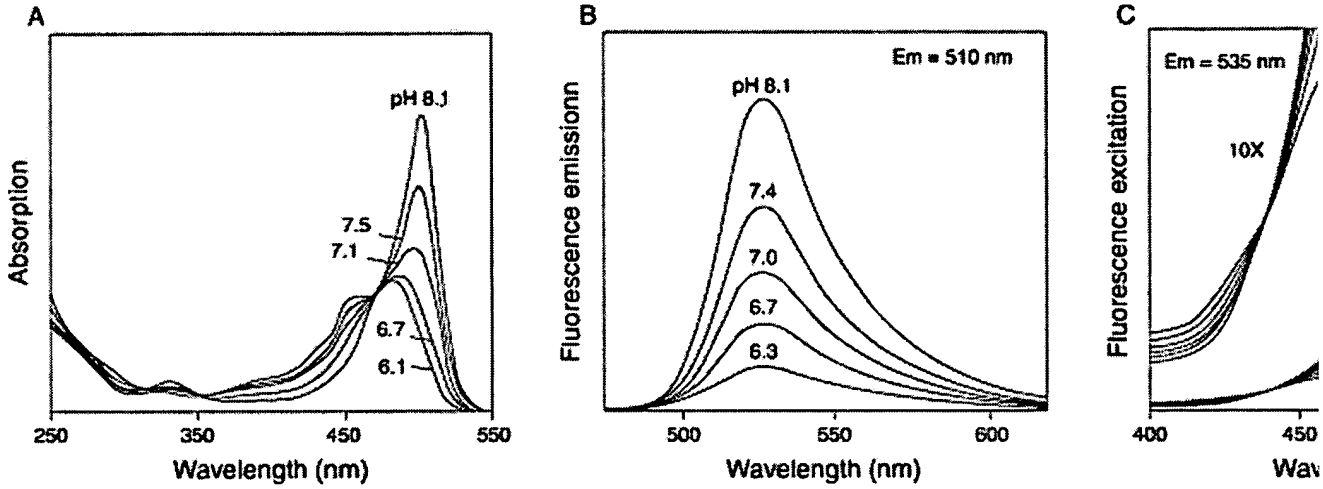
Figure 20.2 The pH-dependent spectra of fluorescein (F1300): **A)** absorption spectra, **B)** emission spectra.





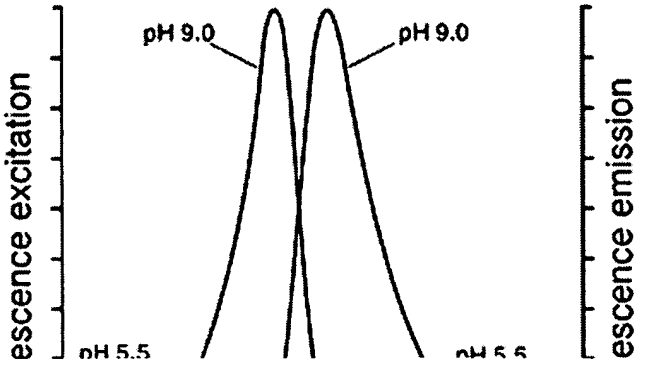
* * * * *

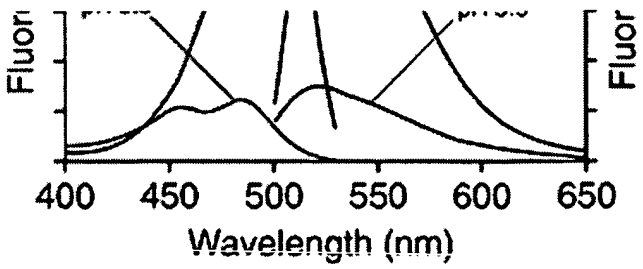
Figure 20.3 The pH-dependent spectra of BCECF (B1151): **A)** absorption spectra, **B)** emission sp excitation spectra. The fluorescence excitation spectra have been enlarged on the left to reveal BC isosbestic point. Note that the isosbestic point of the excitation spectra of BCECF is different from absorption spectra (compare panels A and C).



* * * * *

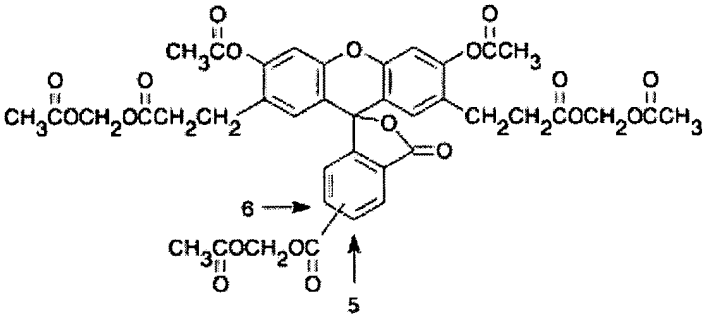
Figure 20.4 Fluorescence excitation (detected at 535 nm) and emission (excited at 490 nm) spectra of BCECF in pH 9.0 and 5.5 buffers.



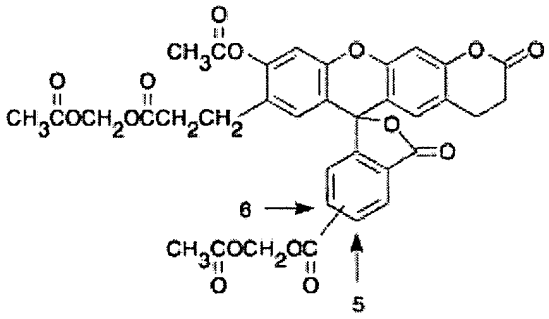


* * * * *

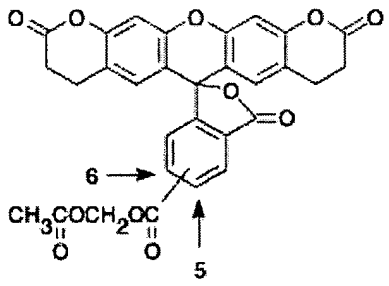
Figure 20.5 Structures of the AM esters of BCECF (B1150, B1170, B3051).



I (Molecular Weight = 820.7)



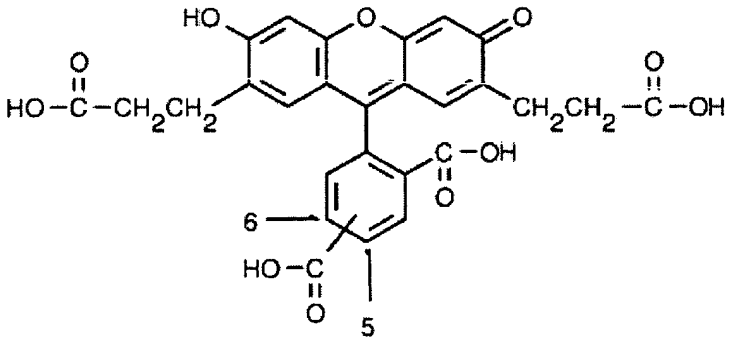
II (Molecular Weight = 688.6)



III (Molecular Weight = 556.5)

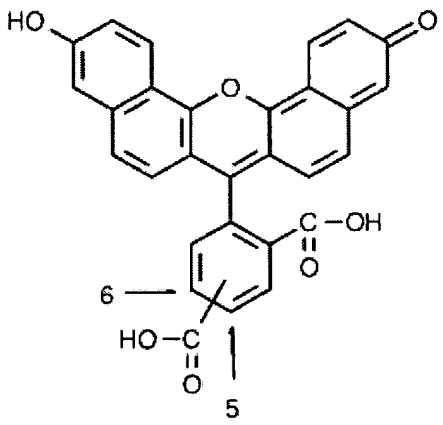
* * * * *

Figure 20.6 B1151 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF acid).



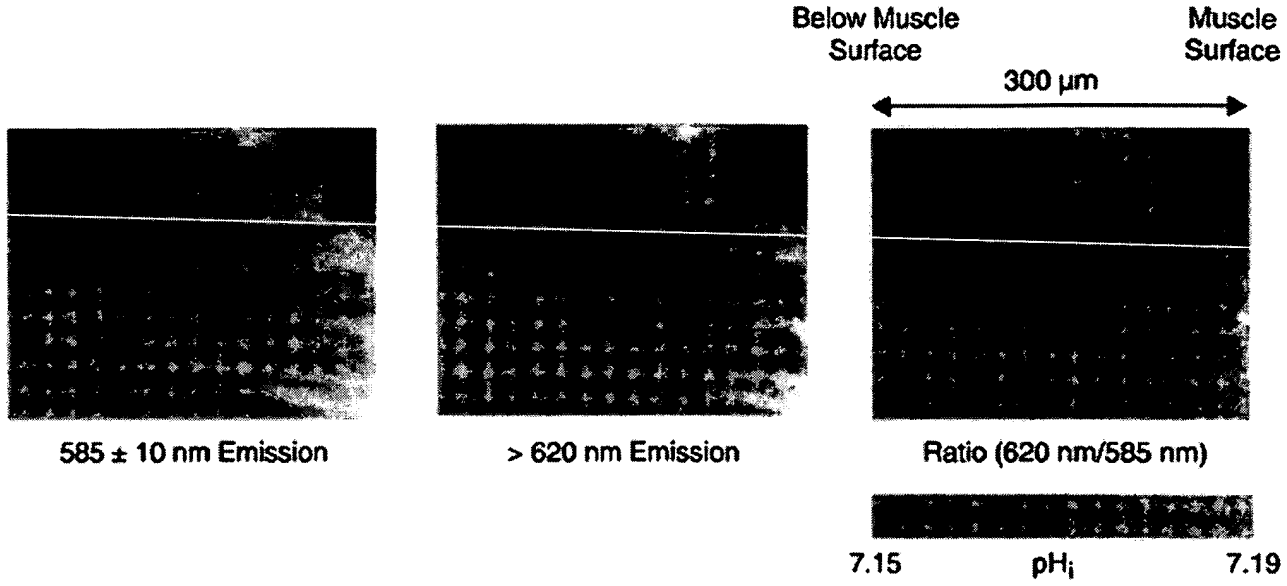
* * * * *

Figure 20.7 C652 5-(and-6)-carboxynaphthofluorescein.



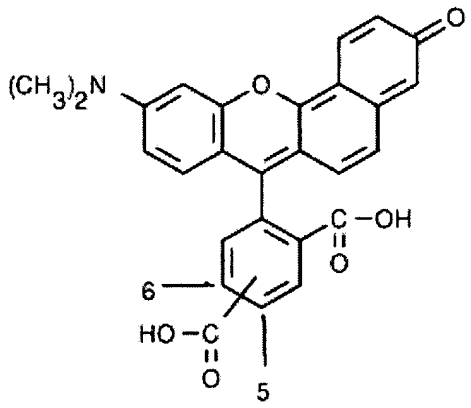
* * * * *

Figure 20.8 Confocal fluorescence images of rabbit papillary muscle loaded by perfusion with carboxy SNARF-1, AM, acetate (C1271, C1272). Images A and B were acquired through 585 ± 10 nm bandpass and >620 nm longpass emission filters, respectively. The 620 nm/585 nm fluorescence ratio image (panel C) is more uniform than the component images A and B due to cancellation of intensity variations resulting from heterogeneous uptake of the fluorescent indicator. Images contributed by Barbara Muller-Borer and John Lemasters, University of North Carolina and reprinted with permission from (Am J Physiol 275, H1937 (1998)).



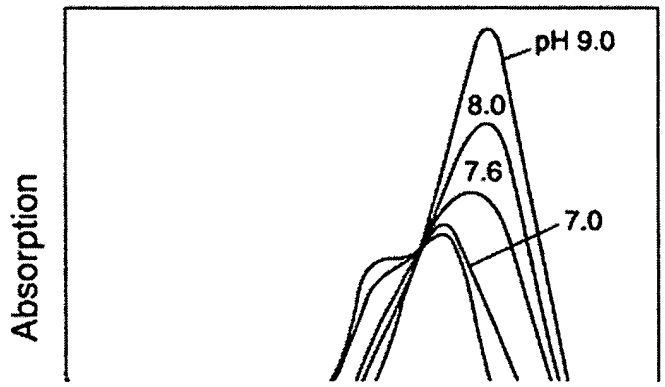
* * * * *

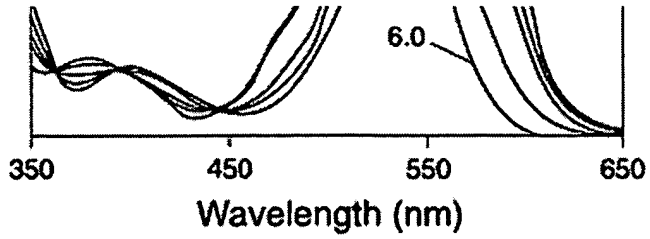
Figure 20.9 C1270 5-(and-6)-carboxy SNARF-1.



* * * * *

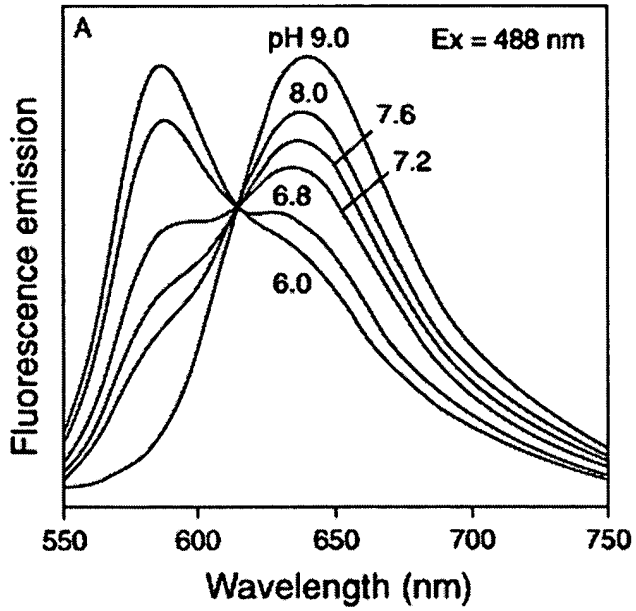
Figure 20.10 The pH-dependent absorption spectra of carboxy SNARF-1 (C1270).

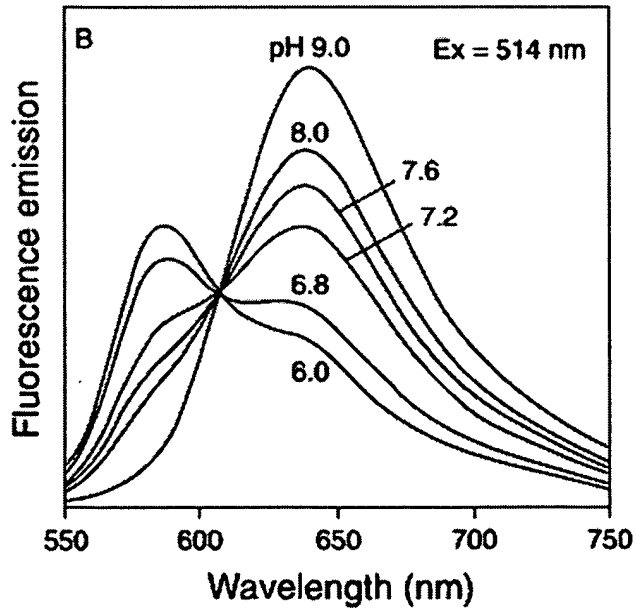


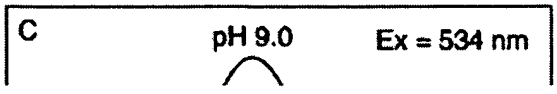


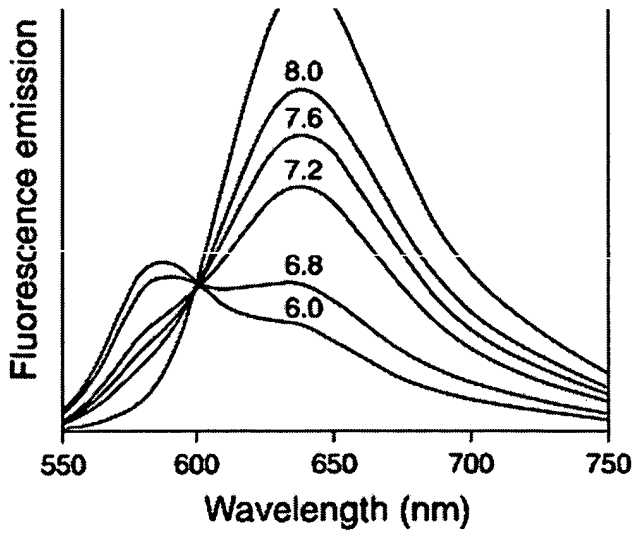
* * * * *

Figure 20.11 The pH-dependent emission spectra of carboxy SNARF-1 (C1270) when it is excited at **A)** 488 nm, **B)** 514 nm and **C)** 534 nm.



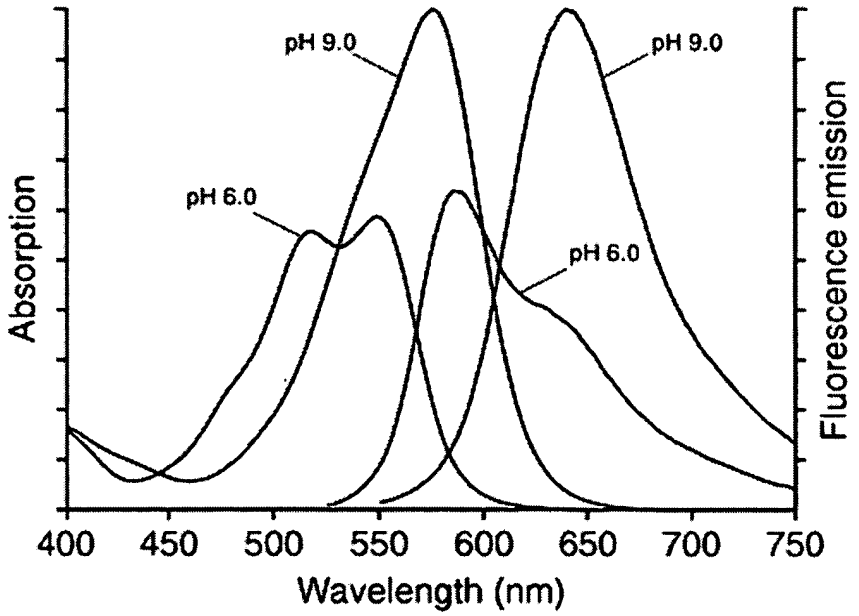






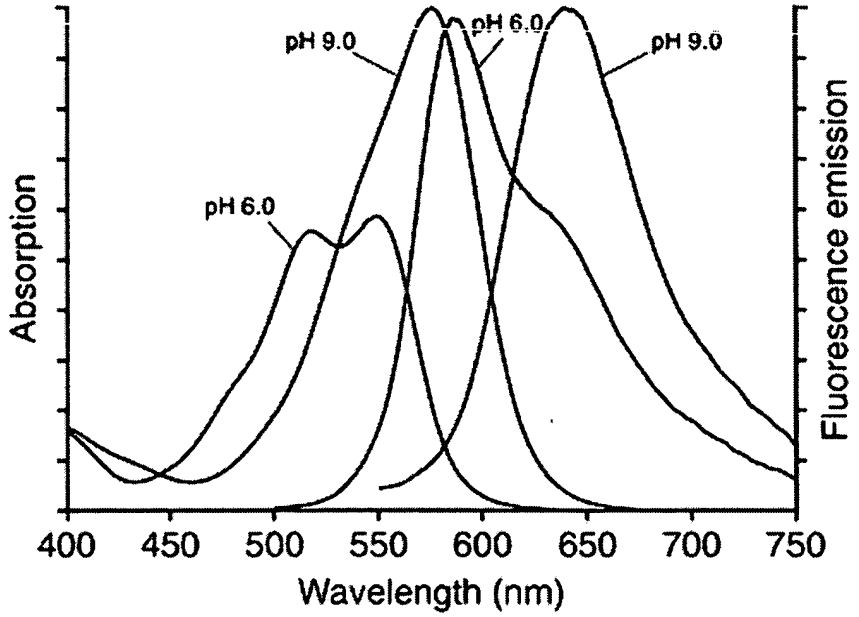
* * * * *

Figure 20.12 Absorption and fluorescence emission (excited at 514 nm) spectra of carboxy SNARF-1 in pH 9.0 and 6.0 buffers.



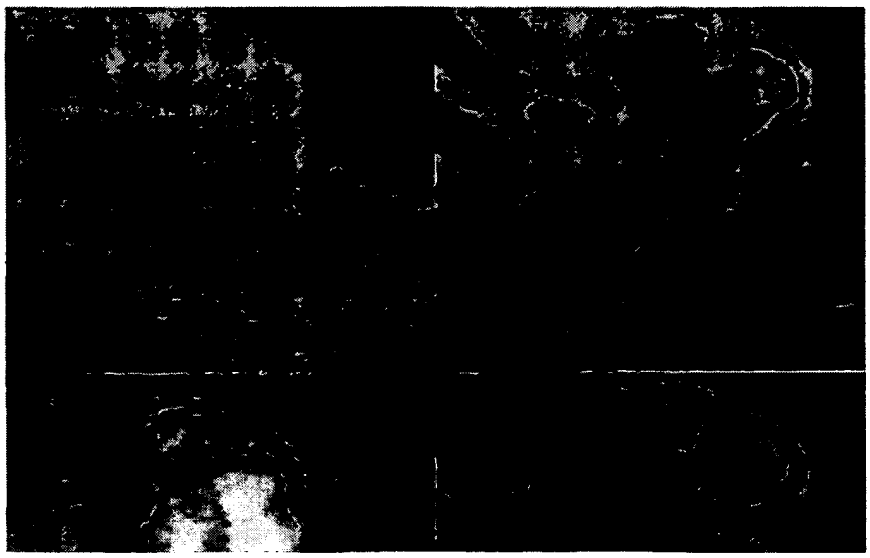
* * * * *

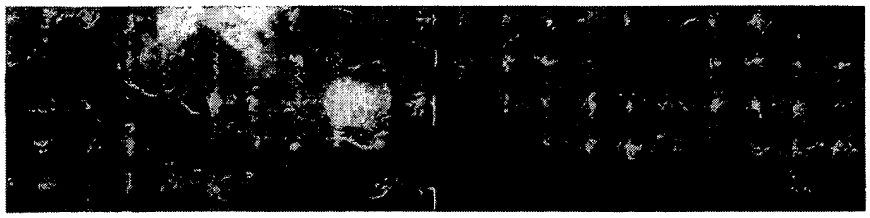
Figure 20.13 Absorption and fluorescence emission (excited at 488 nm) spectra of carboxy SNARF-1 in pH 9.0 and pH 6.0 buffers.



* * * * *

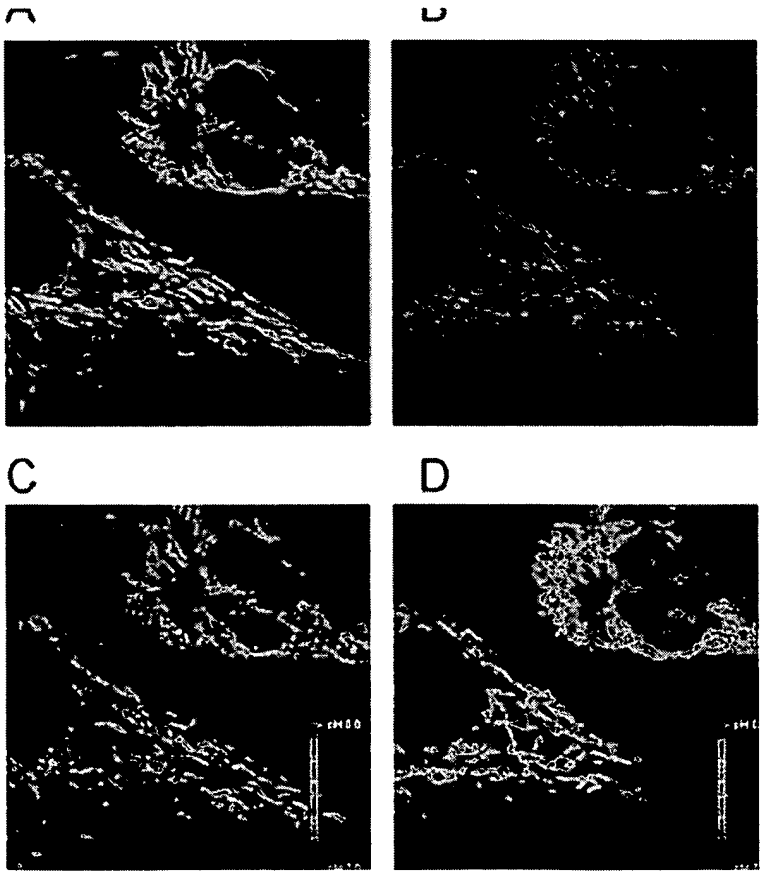
Figure 20.14 Rat pituitary intermediate lobe melanotropes labeled with the indo-1 AM (I1203, I1223, I1226) and carboxy SNARF-1, AM, acetate (C1271, C1272) indicators. Pseudocolored fluorescence from the dual-emission Ca^{2+} indicator, indo-1, is shown at 405 and 475 nm (left panels). Pseudocolored fluorescence from the dual-emission pH indicator, carboxy SNARF-1, is shown at 575 and 640 nm (right panels). Image contributed by Stephen J. Morris, University of Missouri-Kansas City, and Diane M. Beatty, Molecular Probes, Inc.





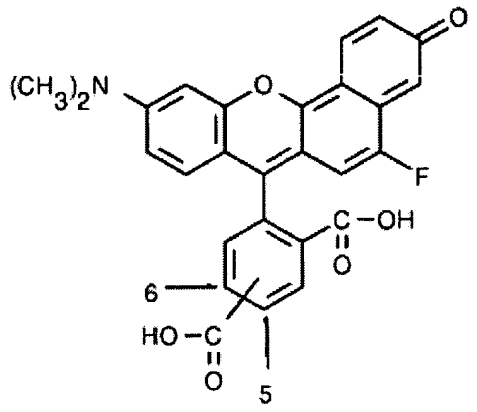
* * * * *

Figure 20.15 Selective loading of carboxy SNARF-1 into mitochondria. BHK cells were loaded with 10 µM carboxy SNARF-1, AM, acetate (C1271, C1272) for 10 minutes, followed by incubation for 4 hours at room temperature. **A**) Confocal image (488 nm excitation) of mitochondrial-selective loading of carboxy SNARF-1 visualized through a 560–600 nm bandpass filter. **B**) Confocal image of the same cells as in **A**, but using a 605 nm dichroic mirror and a 610 nm longpass filter. **C**) Ratio image (**A** and **B**) of mitochondria in cells pseudocolored to represent different pH levels. **D**) Change in mitochondrial pH following the addition of 10 µM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), resulting in a decrease (acidification) of mitochondrial pH. Image contributed by Brian Herman, University of Texas Health Science Center, San Antonio, and reprinted with permission from Biotechniques 30, 804 (2001).



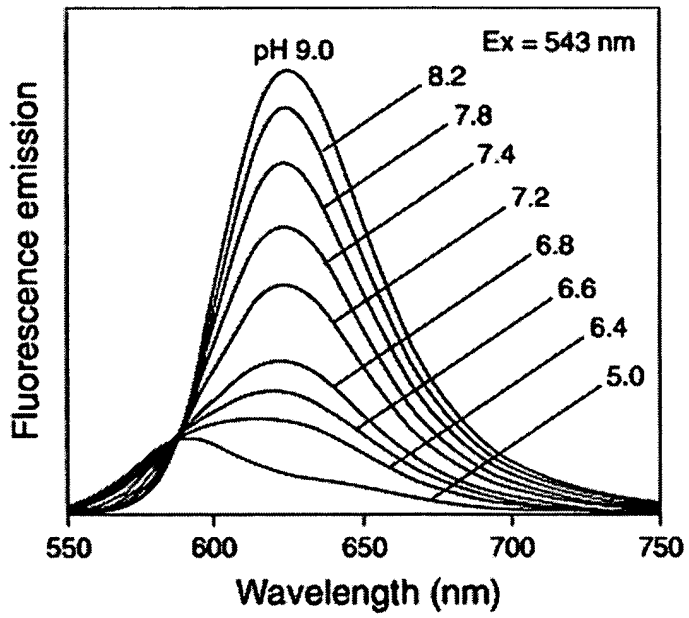
* * * * *

Figure 20.16 S23922 SNARF-5F 5-(and-6)-carboxylic acid.



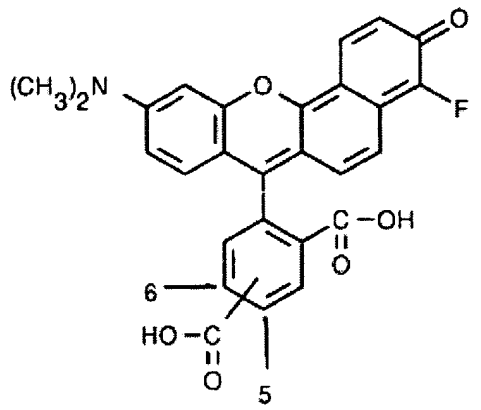
* * * * *

Figure 20.17 Fluorescence emission spectra of SNARF-5F 5-(and 6-)carboxylic acid (S23922) as a function of pH.



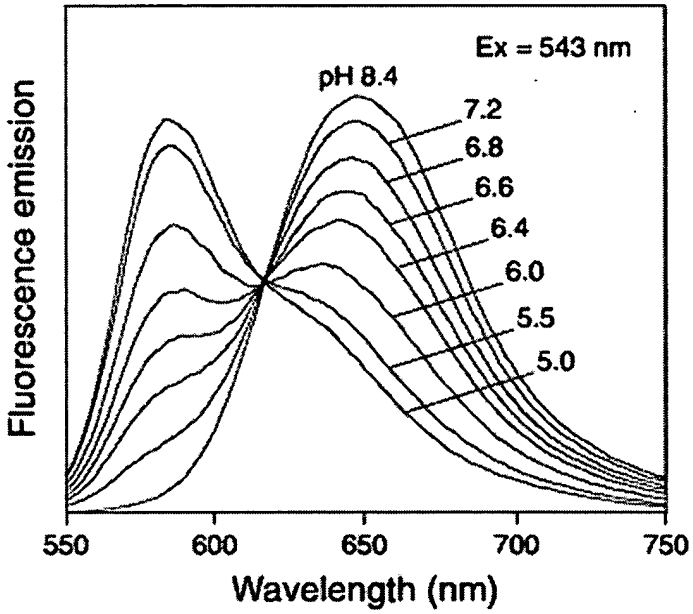
* * * * *

Figure 20.18 S23920 SNARF-4F 5-(and-6)-carboxylic acid.



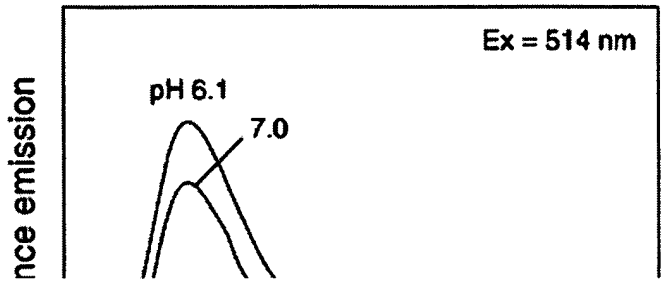
* * * * *

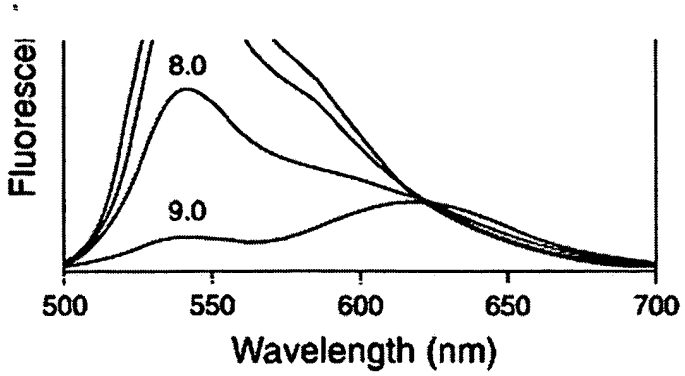
Figure 20.19 Fluorescence emission spectra of SNARF-4F 5-(and 6-)carboxylic acid (S23920) showing the pH-dependent spectral shift that is characteristic of this and other SNARF pH indicators.



* * * * *

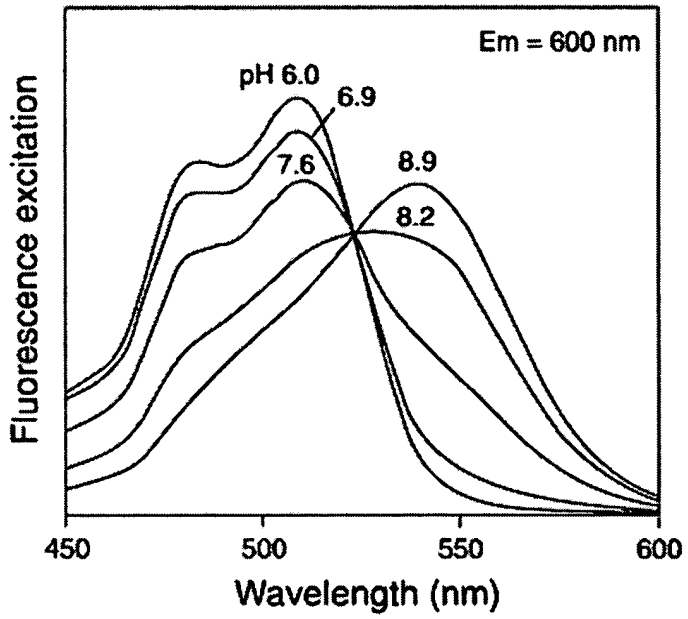
Figure 20.20 The pH-dependent emission spectra of 5-(and 6-)carboxy SNAFL-1 (C1255).





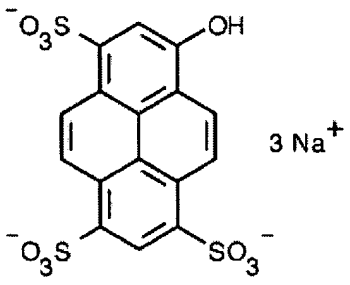
* * * * *

Figure 20.21 The pH-dependent excitation spectra of 5-(and 6-)carboxy SNAFL-1 (C1255) with emission monitored at 600 nm.



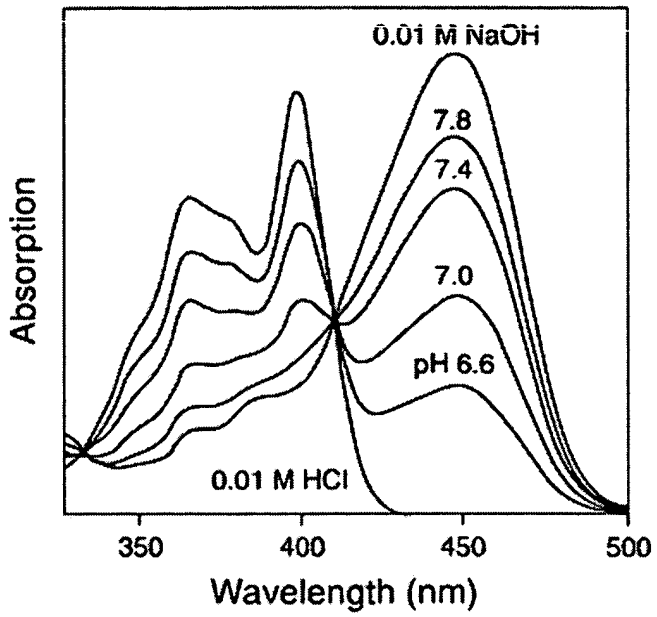
* * * * *

Figure 20.22 H348 8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt (HPTS; pyranine).



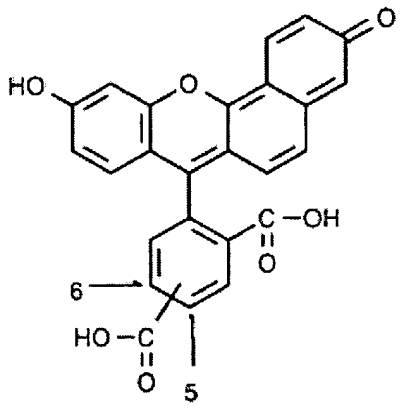
* * * * *

Figure 20.23 The pH-dependent absorption spectra of 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, H348).



* * * * *

Figure. C1255 5-(and-6)-carboxy SNAFL-1.



* * * * *

Product List — 20.2 Probes Useful at Near-Neutral pH

Cat #	Product Name	Unit Size
B1151	2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF acid) *mixed isomers*	1 mg
B1150	2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM)	1 mg
B1170	2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM) *special packaging*	20 x 50 µg
B3051	2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM) *1 mg/mL solution in dry DMSO*	1 mL
B14440 TBD	2',7'-bis-(3-carboxypropyl)-5-(and-6)-carboxyfluorescein (BCPCF acid) *mixed isomers*	1 mg
B14441 TBD	2',7'-bis-(3-carboxypropyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCPCF, AM)	1 mg
C1904	5-(and-6)-carboxyfluorescein (5(6)-FAM) *FluoroPure™ grade* *mixed isomers*	100 mg
C194	5-(and-6)-carboxyfluorescein *mixed isomers*	5 g
C195	5-(and-6)-carboxyfluorescein diacetate (5(6)-CFDA) *mixed isomers*	100 mg
C1354	5-carboxyfluorescein diacetate, acetoxymethyl ester (5-CFDA, AM)	5 mg
C1157	5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (5(6)-CFDA, SE; CFSE) *mixed isomers*	25 mg
C652	5-(and-6)-carboxynaphthofluorescein *mixed isomers*	100 mg
C13196	5-(and-6)-carboxynaphthofluorescein diacetate	10 mg
C1255	5-(and-6)-carboxy SNAFL®-1	1 mg
C1256 TBD	5-(and-6)-carboxy SNAFL®-1, diacetate	1 mg
C1260 TBD	5-(and-6)-carboxy SNAFL®-2	1 mg
C1261 TBD	5-(and-6)-carboxy SNAFL®-2, diacetate	1 mg
C1270	5-(and-6)-carboxy SNARF®-1	1 mg
C1271	5-(and-6)-carboxy SNARF®-1, acetoxymethyl ester, acetate	1 mg
C1272	5-(and-6)-carboxy SNARF®-1, acetoxymethyl ester, acetate *special packaging*	20 x 50 µg
C2925	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate)	1 mg
C7025	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) *special packaging*	20 x 50 µg

C6826	5-(and-6)-chloromethyl SNARF®-1, acetate *mixed isomers* *special packaging*	20 x 50 µg
F1300	fluorescein *reference standard*	1 g
F1303	fluorescein diacetate (FDA)	1 g
F1130	fluorescein-5-(and-6)-sulfonic acid, trisodium salt	100 mg
H348	8-hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt (HPTS; pyranine)	1 g
N1495	nigericin, free acid	10 mg
N7065 TBD	1-(2-nitrophenyl)ethyl phosphate, diammonium salt (NPE-caged phosphate)	5 mg
S23920	SNARF®-4F 5-(and-6)-carboxylic acid	1 mg
S23921	SNARF®-4F 5-(and-6)-carboxylic acid, acetoxymethyl ester, acetate *special packaging*	20 x 50 µg
S23922	SNARF®-5F 5-(and-6)-carboxylic acid	1 mg
S23923	SNARF®-5F 5-(and-6)-carboxylic acid, acetoxymethyl ester, acetate *special packaging*	20 x 50 µg
S1129	5-sulfofluorescein diacetate, sodium salt (SFDA)	25 mg

TBD A product which is to be discontinued; only available as long as current stock remains.

* * * * *

Data Table — 20.2 Probes Useful at Near-Neutral pH

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Abs	EC	Em	So
B1150	~615	F,D	DMSO	<300		none					
B1151	520.45	L	pH >6	482	35,000	520	pH 5	503	90,000	528	pH
B1170	~615	F,D	DMSO	<300		none					
B3051	~615	F,D	DMSO	<300		none					
B14440 TBD	548.50	L	pH >6	483	36,000	520	pH 5	504	92,000	529	pH
B14441 TBD	848.77	F,D	DMSO	<300		none					
C194	376.32	L	pH >6, DMF	475	28,000	517	pH 5	492	75,000	517	pH
C195	460.40	F,D	DMSO	<300		none					
C652	476.44	L	pH >6, DMF	512	11,000	563	pH 6	598	49,000	668	pH
C1157	557.47	F,D	DMF, DMSO	<300		none					

C1255	426.38	L	pH >6	508	29,000	543	pH 6	540	52,000	623	pH
C1256 TBD	510.46	F,D	DMSO	<350		none					
C1260 TBD	460.83	L	pH >6	514	31,000	546	pH 6	543	50,000	630	pH
C1261 TBD	544.90	F,D	DMSO	<350		none					
C1270	453.45	L	pH >6	548	27,000	587	pH 6	576	48,000	635	pH
C1271	567.55	F,D	DMSO	<350		none					
C1272	567.55	F,D	DMSO	<350		none					
C1354	532.46	F,D	DMSO	<300		none					
C1904	376.32	L	pH >6, DMF	475	29,000	517	pH 5	492	78,000	517	pH
C2925	464.86	F,D	DMSO	<300		none					
C6826	499.95	F,D	DMSO	<350		none					
C7025	464.86	F,D	DMSO	<300		none					
C13196	560.52	F,D	DMSO	<300		none					
F1130	478.32	D,L	H_2O, DMF	476	31,000	519	pH 5	495	76,000	519	pH
F1300	332.31	L	pH >6, DMF	473	34,000	514	pH 5	490	93,000	514	pH
F1303	416.39	F,D	DMSO	<300		none					
H348	524.37	D,L	H_2O	403	20,000	511	pH 4	454	24,000	511	pH
N1495	724.97	F,D	MeOH	<300		none					
N7065 TBD	281.20	F,D,LL	H_2O	259	5,700	none	MeOH				
S1129	518.43	F,D	DMSO	<300		none					
S23920	471.44	L	pH >6	552	27,000	589	pH 5	581	48,000	652	pH
S23921	585.54	F,D	DMSO	<350		none					
S23922	471.44	L	pH >6	555	27,000	590	pH 5	579	49,000	630	pH
S23923	585.54	F,D	DMSO	<350		none					

TBD A product which is to be discontinued; only available as long as current stock remains.

Notes

1. MW value is approximate. BCECF, AM is a mixture of molecular species. Lot-specific average MW values are printed on product labels.
2. pK_a values may vary considerably depending on the temperature, ionic strength, viscosity, protein binding and other factors. Unless otherwise noted, values listed have been determined from pH-dependent fluorescence measurements at 22°C.
3. Spectra are in aqueous buffers adjusted to >1 pH unit above and >1 pH unit below the pK_a.
4. This product is supplied as a ready-made solution in the solvent indicated under "Soluble."
5. Data on pH-dependence of C652 spectra obtained at Molecular Probes. Additional relevant data are reported elsewhere (Mikrochim Acta 108, 133 (1992)).
6. Values of pK_a for these SNAFL and SNARF indicators are as reported in published references (Anal Biochem 194, 330 (1991)).
7. This product is specified to equal or exceed 98% analytical purity by HPLC.
8. Acetate hydrolysis of this compound yields a fluorescent product with similar pH-dependent spectral characteristics to C1904.
9. C6826 and S22801 are converted to fluorescent products with spectra similar to C1270 after acetate hydrolysis.
10. The pK_a for H348 was determined in 0.066 M phosphate buffers at 22°C (Fresenius Z Anal Chem 314, 119 (1983)).
11. All photoactivatable probes are sensitive to light. They should be protected from illumination except when photolysis is intended.
12. This compound has weaker visible absorption at >300 nm but no discernible absorption peaks in this region.

* * * * *

Section 20.4 — pH Indicator Conjugates

This section includes our selection of pH indicators conjugated to dextrans and lipids, as well as our chemically reactive pH indicators for preparing new pH-sensitive conjugates.

pH Indicator Dextrans

The pH-sensitive properties of the pH indicators described in Section 20.2 and Section 20.3 are usually not significantly affected upon conjugation to dextrans. However, coupling of pH indicators to these relatively inert polysaccharides changes several other properties of the dyes:

- The conjugates have high water solubility and therefore must be loaded into cells by microinjection, patch-clamp techniques, endocytosis or liposome fusion or by using our Influx pinocytic cell loading reagent (I14402, Section 19.8).
- Once loaded, the dextrans are retained in viable cells for long periods and (at least those dextrans with average molecular weights above 3000) will not pass through gap junctions.
- Attachment to a dextran significantly decreases the likelihood that the indicator will become compartmentalized, thereby avoiding a substantial problem associated with cell-permeant acetoxymethyl (AM) ester derivatives.[1]

The properties of some of the most useful pH indicator dextrans available from Molecular Probes are listed in Table 20.3 in approximate order of decreasing pK_a values. Our numerous labeled dextrans are discussed in Section 14.5 (Table 14.4).

Indicator Dextrans for Measuring Near-Neutral pH

Our 10,000 MW and 70,000 MW BCECF dextrans (D1878, D1880) are important dual-excitation pH indicator conjugates for pH measurements near pH 7.0. BCECF dextran–labeled Swiss 3T3 cells have been shown to produce much more stable fluorescent signals, reduced probe compartmentalization and 10-fold greater resistance to light-induced damage when compared with BCECF AM–labeled cells.[2] The 10,000 MW BCECF dextran (D1878) has been used to monitor intracellular pH increases during developmental processes [3–5] and for pH measurements in submucosal gland secretions from human lung tissues.[6] Cytoplasmic Ca^{2+}/H^+ buffering in green algae has been investigated using BCECF dextran in combination with fura dextran [7] (F3029, Section 19.4).

A dextran conjugate of the carboxy SNARF-1 pH indicator (D3303, D3304) has been microinjected into rhizoid cells of the alga *Pelvetia fastigata* and used with ratiometric imaging to measure pH gradients associated with polar tip growth.[8] SNARF-1 dextran has also been used to detect cytosolic alkalinization associated with multidrug transporter activity [9] and to investigate pH regulation of connexin 43 channels.[10] SNARF dextran conjugates have been scrape-loaded into the cytosol of MDF-7/ADR cells. It was found that the 70,000 MW SNARF dextran conjugate remained exclusively cytosolic, whereas the 10,000 MW conjugate reported the pH of both cytosolic and nuclear compartments.[11]

The absorption and emission spectra of HPTS dextran (D7179) are shifted to longer wavelengths by about 20 nm relative to those of free HPTS (Figure 20.23). Also, due to a change in the shape of the spectrum, the absorption maximum of the acid form in aqueous solution (~375 nm) is actually shorter than that of the free dye (~403 nm). HPTS dextran retains the ratiometric

capabilities of the parent fluorophore discussed in Section 20.2 and exhibits a pK_a of almost exactly 7.0.

Indicator Dextrans for Measuring Acidic pH

Although the fluorescein, BCECF, HPTS and SNARF dextrans are intended for pH measurements between pH ~6 and 8, these dextrans are also useful for detecting uptake into acidic organelles, such as occurs during endocytosis. In particular, when these indicator dextrans enter moderately acidic compartments (pH <5.5):

- Fluorescence of the fluorescein, BCECF and HPTS dextrans is strongly quenched [12-14] (Figure 20.2, Figure 20.3).
- The 520/570 nm emission intensity ratio of the double-labeled fluorescein–tetramethylrhodamine dextrans (D1950, D1951) decreases [15] (Figure 20.32).
- The 580/640 nm emission ratio of the SNARF-1 dextrans increases (Figure 20.11).

The indicator dextrans discussed above are useful for detecting translocation into compartments that have an acidic pH; however, the relative insensitivity of their fluorescence below pH ~6 limits quantitative pH estimation. The lower pK_a values of our Oregon Green 488 and Oregon Green 514 dextran conjugates (Table 20.3) make them more suitable indicators for estimating the pH of relatively acidic lysosomal environments. Moreover, the shift in their excitation spectra in acidic media permits ratiometric pH measurements.

We have also developed a 10,000 MW dextran conjugate of the LysoSensor Yellow/Blue dye (L22460), which can be used to quickly and accurately measure the pH of lysosomes. As this labeled dextran is taken up by the cells and moves through the endocytic pathway, the fluorescence of the LysoSensor dye changes from blue fluorescent in the near-neutral endosomes to longer-wavelength yellow fluorescent in the acidic lysosomes.[16] The greatest change in fluorescence emission occurs near the pK_a of the dye at pH ~4.2. The pH in lysosomes can be measured with LysoSensor Yellow/Blue dextran using fluorescence microscopy (Figure 12.37) or flow cytometry.

Lipophilic pH Indicators

Lipophilic Fluoresceins

Measurement of the pH adjacent to membrane surfaces is often complicated by electrostatic charge and solvation effects on the pK_a of surface-bound indicators.[17,18] The pK_a of membrane-intercalated fluorescein DHPE (F362) is ~6.2, quite close to that of free fluorescein.[17] Researchers have used the pH-dependent fluorescence of fluorescein DHPE to measure lateral proton conduction along lipid monolayers.[19-22] This fluorescein-labeled phospholipid has also been used to follow proton translocation from internal compartments in phospholipid vesicles.[23] For more acidic environments, Oregon Green 488 DHPE (O12650, Figure 20.33) has potentially similar applications. Other related lipophilic fluorescein derivatives, including 5-dodecanoylaminofluorescein (D109, Figure 20.34), 5-hexadecanoylaminofluorescein (H110) and 5-octadecanoylaminofluorescein (O322), are described in Section 13.5.

Lipophilic Coumarins

4-Heptadecyl-7-hydroxycoumarin (H22730) is an alkyl derivative of the pH-sensitive blue-fluorescent 7-hydroxycoumarin (umbelliferone) fluorophore (Figure 13.48). As with other lipophilic coumarins,[24] 4-heptadecyl-7-hydroxycoumarin is primarily useful as a probe of membrane surfaces. Deprotonation of the 7-hydroxyl group is expected to be strongly

dependent on membrane-surface electrostatic potential. The pK_a of 4-heptadecyl-7-hydroxycoumarin varies from 6.35 in the cationic detergent CTAB to 11.15 in the anionic detergent sodium dodecyl sulfate (SDS), as measured by its fluorescence response.[18] The pK_a values of lipophilic pH indicators are strongly dependent on the ionic composition of the membrane surface,[18,25] making them sensitive probes of membrane-surface electrostatic potential.[26–28] 4-Heptadecyl-7-hydroxycoumarin has been used to measure pH differences at membrane interfaces in isolated plasma membranes of normal and multidrug-resistant murine leukemia cells,[29,30] and has also been employed to characterize interactions of cationic lipids with DNA.[26,27,31]

Reactive Dyes for Preparing pH-Sensitive Conjugates

Many of the pH indicators described in Section 20.2 and Section 20.3 can be conjugated to biological molecules in order to generate pH-sensitive tracers. The resulting conjugates can be used to follow endocytosis, phagocytosis, organelle trafficking and other processes, as described in Section 16.1. For example, the pH sensitivity of fluorescein-labeled transferrin (T2871, Section 16.1) has frequently been exploited to detect pH changes associated with the endocytic processing of this important iron-transporting glycoprotein.[32–35] For this type of application, fluorescein conjugates are less than optimal because they have little sensitivity in the pH range below pH 5.5. The response range can be extended using conjugates of our Oregon Green 488 dye, including Oregon Green 488 transferrin (T13341, Section 16.1), which has a much lower pK_a than fluorescein (4.7 compared to 6.4, Figure 1.12) but essentially identical spectra. Our collaborators, Dr. Elizabeth Simons and her co-workers, have labeled fungi (*Cryptococcus neoformans*) with FITC (F143, F1906, F1907; Section 1.5) or Oregon Green 488 isothiocyanate (O6080, Section 1.5) and used them to study the influence of phagosomal pH on the fungicidal and fungistatic activity of human monocyte–derived macrophages.[36] At pH levels evoked by phagocytosis of live and heat-killed fungi (pH 4.7–5.7), standard curves of the 498/450 nm fluorescence excitation ratio as a function pH (Figure 20.35) illustrate the greater sensitivity of the Oregon Green 488 conjugates. Vergne and co-workers have used zymosan (heat-killed yeast) double-labeled with the succinimidyl esters of Oregon Green 488 carboxylic acid (O6147) and carboxytetramethylrhodamine (C1171, Section 1.6) to measure phagosomal pH in J774 macrophages. Using dual-emission (530/585 nm) flow cytometry, they were able to estimate pH values as low as 4.0.[37]

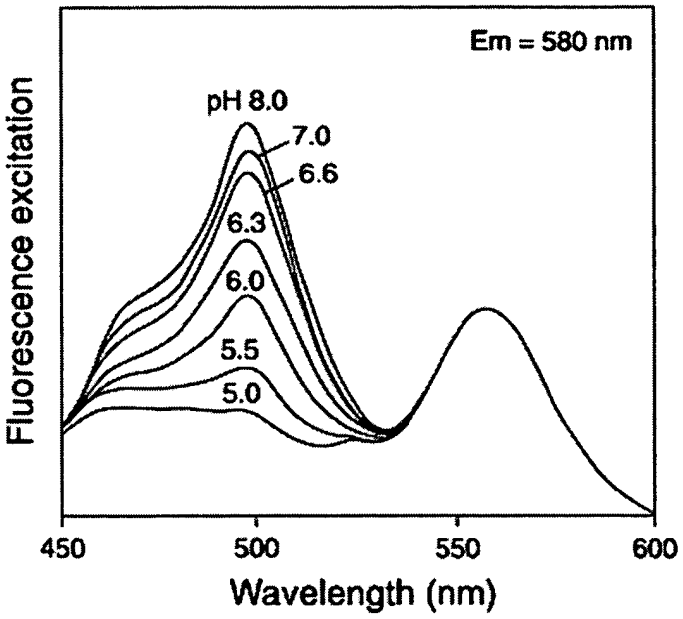
Most of the reagents or methods required to prepare conjugates have been described in Chapter 1. The most common method of producing a useful conjugate is the reaction of amines with succinimidyl esters or isothiocyanates of the pH indicator. Examples include the succinimidyl esters of the carboxy SNARF-1, carboxy SNAFL-1 and carboxynaphthofluorescein dyes (S22801, C3061, C653). A heptapeptide containing a peroxisomal targeting signal was conjugated to the carboxy SNAFL-2 succinimidyl ester to produce a pH-sensitive peptide that was also cell permeant and localized in peroxisomes; a pH-insensitive peptide was also synthesized using the BODIPY 581/591 succinimidyl ester.[38] The succinimidyl esters of the chlorinated fluorescein derivatives 6-JOE (pK_a ~11.5, C6171MP; Section 1.5), 6-TET (pK_a ~4.5, C20092; Section 1.5) and 6-HEX (pK_a ~3, C20091; Section 1.5) can be used to prepare conjugates that are responsive to strongly acidic (6-HEX) or alkaline (6-JOE) pH levels. When the amine-reactive pH indicator is not available, sulfosuccinimidyl esters can generally be prepared *in situ* simply by dissolving the carboxylic acid dye in a buffer that contains *N*-hydroxysulfosuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (NHSS, H2249; EDAC, E2247; Section 3.3). Addition of NHSS to the buffer has been shown to enhance the yield of carbodiimide-mediated conjugations.[39] Suitable amine-reactive pH indicators or dyes that can be made reactive using EDAC/NHSS are listed in Table 20.4.

1. J Exp Biol 196, 419 (1994); **2.** J Cell Physiol 141, 410 (1989); **3.** Dev Biol 191, 53 (1997); **4.** Development 120, 433 (1994); **5.** Dev Biol 156, 176 (1993); **6.** Proc Natl Acad Sci U S A 98, 8119 (2001); **7.** Protoplasma 198, 107 (1997); **8.** Science 263, 1419 (1994); **9.** J Histochem

Cytochem 38, 685 (1990); **10.** Biophys J 70, 1294 (1996); **11.** Proc Natl Acad Sci U S A 96, 4432 (1999); **12.** Proc Natl Acad Sci U S A 91, 4811 (1994); **13.** J Biol Chem 268, 25320 (1993); **14.** J Cell Sci 105, 861 (1993); **15.** J Cell Biol 130, 821 (1995); **16.** Nucleic Acids Res 30, 1338 (2002); **17.** Biochim Biophys Acta 939, 289 (1988); **18.** J Phys Chem 81, 1755 (1977); **19.** Biochemistry 29, 59 (1990); **20.** J Am Chem Soc 113, 8818 (1991); **21.** Eur J Biochem 162, 379 (1987); **22.** Nature 322, 756 (1986); **23.** Biochim Biophys Acta 766, 161 (1984); **24.** Biochim Biophys Acta 1284, 191 (1996); **25.** Biochim Biophys Acta 323, 326 (1973); **26.** Biochim Biophys Acta 1464, 251 (2000); **27.** Biochim Biophys Acta 1368, 115 (1998); **28.** Methods Enzymol 171, 376 (1989); **29.** Biochemistry 29, 7275 (1990); **30.** Biochim Biophys Acta 729, 185 (1983); **31.** Biochim Biophys Acta 1419, 207 (1999); **32.** Biochemistry 31, 5820 (1992); **33.** Cell 37, 789 (1984); **34.** J Biol Chem 266, 3469 (1991); **35.** J Biol Chem 265, 6688 (1990); **36.** Infect Immun 67, 885 (1999); **37.** Anal Biochem 255, 127 (1998); **38.** Histochem J 33, 65 (2001); **39.** Anal Biochem 156, 220 (1986).

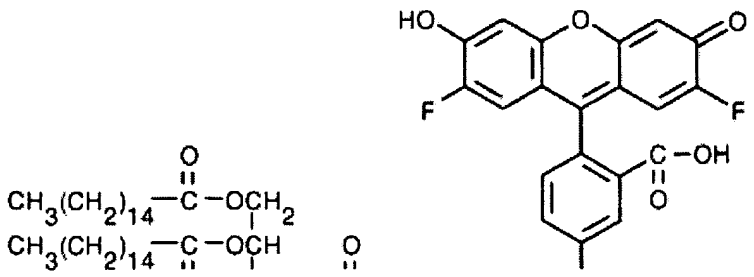
* * * * *

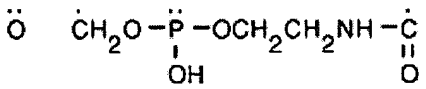
Figure 20.32 The excitation spectra of double-labeled fluorescein–tetramethylrhodamine dextran (D1950, D1951), which contains pH-dependent (fluorescein) and pH-independent (tetramethylrhodamine) dyes.



* * * * *

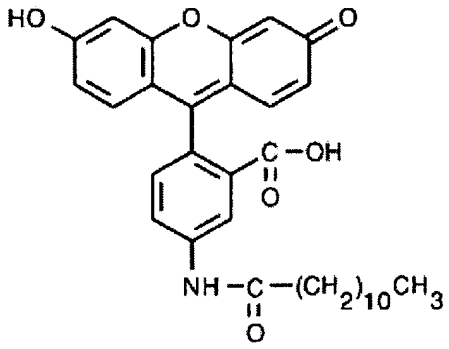
Figure 20.33 O12650 Oregon Green 488 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Oregon Green 488 DHPE).





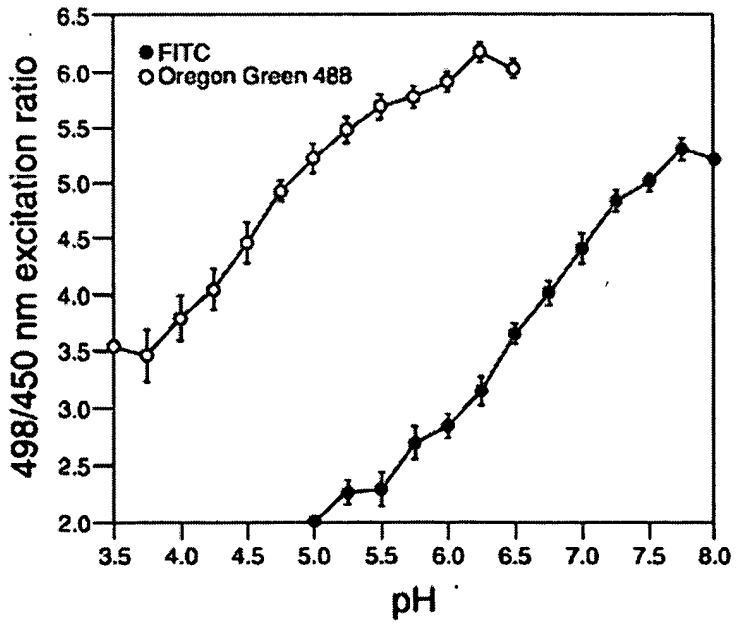
* * * * *

Figure 20.34 D109 5-dodecanoylaminofluorescein.



* * * * *

Figure 20.35 Calibration curves for intraphagosomal pH measurements using fungi *(Cryptococcus neoformans)* labeled with Oregon Green 488 isothiocyanate (O6080) or fluorescein isothiocyanate (FITC, F143, F1906, F1907). Human monocyte-derived macrophages laden with phagocytosed *C. neoformans* were exposed to pH-controlled buffers in the presence of the K^+/H^+ ionophore nigericin (N1495). The 498/450 nm fluorescence excitation ratios corresponding to different pH levels were measured in a spectrofluorometer. The data were provided by Dr. Elizabeth Simons, Boston University.



* * * * *

Table 20.3 — Molecular Probes' pH indicator dextrans, in order of decreasing pK_a

Dye	Cat #	pK_a *	Measurement Wavelengths	Application Notes
SNARF	D3303, D3304	~7.5	Emission ratio 580/640 nm excited at 514 or 488 nm	• Best conjugate for ratiometric emission measurements, with spectra similar to carboxy SNARF-1 (Figure 20.11)
HPTS	D7179	~7.0	Excitation ratio 470/380 nm detected at 530 nm	• Spectra of dextran conjugate are significantly shifted (~20 nm) relative to free dye
BCECF	D1878, D1880	~7.0	Excitation ratio 490/440 nm detected at 530 nm	• Best conjugate for ratiometric excitation measurements, with spectra similar to BCECF (Figure 20.3)
Fluorescein	D1821, D1823, D1844, D3305	~6.4	Excitation ratio 490/450 nm detected at 520 nm	• Fluorescence is strongly quenched upon uptake into acidic organelles (Figure 20.2)
Fluorescein and tetramethylrhodamine	D1950, D1951	~6.4	Excitation ratio 495/555 nm detected at 580 nm †	• Conjugate incorporating both pH-sensitive and pH-insensitive fluorescent dyes (Figure 20.32)
Oregon Green 488	D7170, D7172	~4.7	Excitation ratio 490/440 nm detected at 520 nm	• Good photostability • Optimum pH sensitivity for measurements in lysosomes and late endosomes

Oregon Green 514	D7176	~4.7	Excitation ratio 510/450 nm detected at 530 nm	• Excellent photostability • Optimum pH sensitivity for measurements in lysosomes and late endosomes (Figure 20.30)
LysoSensor Yellow/Blue	L22460	~4.2	Excitation ratio 340/400 nm detected at 520 nm; emission ratio 450/510 nm excited at 365 nm	• Options for dual excitation or dual emission ratio measurements • Optimum pH sensitivity for measurements in lysosomes and late endosomes
* pK_a values are those determined for the free dyes. Actual values for dextran conjugates may differ by up to +/– 0.3 pH units and may vary with production lots. † Ratiometric emission measurements at 520/580 nm (with excitation at 495 nm) are also possible in principle; however, the response may be complicated by fluorescence resonance energy transfer.				

* * * * *

Table 20.4 — Reactive pH indicator dyes

pH Indicator	Preferred Reactive Form
BCECF	BCECF (B1151, Section 20.2) *
Carboxyfluorescein	5-(and 6-)carboxyfluorescein, succinimidyl ester (C1311, Section 1.5)
6-HEX	6-carboxy-2',4,4',5',7,7'-hexachlorofluorescein, succinimidyl ester (C20091, Section 1.5)
6-JOE	6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein, succinimidyl ester (C6171MP, Section 1.5)
Naphthofluorescein	5-(and 6-)carboxynaphthofluorescein, succinimidyl ester (C653)
Oregon Green 488	Oregon Green 488 carboxylic acid, succinimidyl ester (O6147, O6149)
Oregon Green 514	Oregon Green 514 carboxylic acid, succinimidyl ester (O6139)
SNAFL-1	5-(and 6-)carboxy SNAFL-1, succinimidyl ester (C3061)
SNARF-1	SNARF-1 carboxylic acid, acetate, succinimidyl ester (S22801); 5-(and 6-)carboxy SNARF-1 (C1270, Section 20.2) *
6-TET	6-carboxy-2',4,7,7'-tetrachlorofluorescein, succinimidyl ester (C20092, Section 1.5)

* Carboxylic acids require activation with EDAC/NHSS before reaction with amines (Section 1.1).

* * * * *

Product List — 20.4 pH Indicator Conjugates

Cat #	Product Name	Unit Size
C653	5-(and-6)-carboxynaphthofluorescein, succinimidyl ester *mixed isomers*	25 mg
C3061 *TBD*	5-(and-6)-carboxy SNAFL®-1, succinimidyl ester *mixed isomers*	1 mg
C3062 *TBD*	5-(and-6)-carboxy SNAFL®-2, succinimidyl ester *mixed isomers*	1 mg
D1878	dextran, BCECF, 10,000 MW, anionic	10 mg
D1879 *TBD*	dextran, BCECF, 40,000 MW, anionic	10 mg
D1880	dextran, BCECF, 70,000 MW, anionic	10 mg
D12980 *TBD*	dextran, 4',5'-dichloro-2',7'-dimethoxyfluorescein, 10,000 MW, anionic	5 mg
D12981 *TBD*	dextran, 4',5'-dichloro-2',7'-dimethoxyfluorescein, 70,000 MW, anionic	5 mg
D3305	dextran, fluorescein, 3000 MW, anionic	10 mg
D1821	dextran, fluorescein, 10,000 MW, anionic	25 mg
D1844	dextran, fluorescein, 40,000 MW, anionic	25 mg
D1823	dextran, fluorescein, 70,000 MW, anionic	25 mg
D1950 *TBD*	dextran, fluorescein and tetramethylrhodamine, 10,000 MW, anionic	10 mg
D1951	dextran, fluorescein and tetramethylrhodamine, 70,000 MW, anionic	10 mg
D7179	dextran, 8-hydroxypyrene-1,3,6-trisulfonic acid, 10,000 MW, anionic (HPTS dextran)	5 mg
D7170	dextran, Oregon Green® 488; 10,000 MW, anionic	5 mg
D7172	dextran, Oregon Green® 488; 70,000 MW, anionic	5 mg
D7174 *TBD*	dextran, Oregon Green® 514; 10,000 MW, anionic	5 mg
D7176	dextran, Oregon Green® 514; 70,000 MW, anionic	5 mg
D3303	dextran, SNARF®-1, 10,000 MW, anionic	5 mg
D3304	dextran, SNARF®-1, 70,000 MW, anionic	5 mg
D12760 *TBD*	6,8-difluoro-4-heptadecyl-7-hydroxycoumarin (C_{17}DiFU)	10 mg
F362	*N*-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (fluorescein DHPE)	5 mg
H22730	4-heptadecyl-7-hydroxycoumarin	10 mg

L22460	LysoSensor™ Yellow/Blue dextran, 10,000 MW, anionic, fixable	5 mg
O6147	Oregon Green® 488 carboxylic acid, succinimidyl ester *5-isomer*	5 mg
O6149	Oregon Green® 488 carboxylic acid, succinimidyl ester *6-isomer*	5 mg
O12650	Oregon Green® 488 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Oregon Green® 488 DHPE)	1 mg
O6139	Oregon Green® 514 carboxylic acid, succinimidyl ester	5 mg
S22801	SNARF®-1 carboxylic acid, acetate, succinimidyl ester *special packaging*	10 x 50 µg

TBD A product which is to be discontinued; only available as long as current stock remains.

* * * * *

Data Table — 20.4 pH Indicator Conjugates

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Abs	EC	Em
C653	573.51	F,D,L	DMF, DMSO	515	10,000	565	pH 6	602	42,000	672
C3061 *TBD*	523.45	F,D,L	DMSO	510	26,000	545	pH 6	542	47,000	625
C3062 *TBD*	557.90	F,D,L	DMSO	520	29,000	545	pH 6	550	47,000	632
D1821	see Notes	F,L	H_2O	473	ND	514	pH 5	490	ND	513
D1823	see Notes	F,L	H_2O	473	ND	514	pH 5	490	ND	514
D1844	see Notes	F,L	H_2O	473	ND	514	pH 5	490	ND	514
D1878	see Notes	F,L	H_2O	482	ND	520	pH 5	503	ND	528
D1879 *TBD*	see Notes	F,L	H_2O	482	ND	520	pH 5	503	ND	528
D1880	see Notes	F,L	H_2O	482	ND	520	pH 5	503	ND	528
D1950 *TBD*	see Notes	F,L	H_2O	see Notes		see Notes				
D1951	see Notes	F,L	H_2O	see Notes		see Notes				
D3303	see Notes	F,L	H_2O	548	ND	587	pH 6	576	ND	635
D3304	see Notes	F,L	H_2O	548	ND	587	pH 6	576	ND	635

D3305	see Notes	F,L	H_2O	473	ND	514	pH 5	490	ND	514
D7170	see Notes	F,L	H_2O	478	ND	518	pH 3	492	ND	518
D7172	see Notes	F,L	H_2O	478	ND	518	pH 3	492	ND	518
D7174 *TBD*	see Notes	F,L	H_2O	489	ND	526	pH 3	506	ND	526
D7176	see Notes	F,L	H_2O	489	ND	526	pH 3	506	ND	526
D7179	see Notes	F,L	H_2O	375	ND	536	pH 5	469	ND	532
D12760 *TBD*	436.58	L	DMSO, EtOH	326	13,000	387	MeOH/H^+	366	15,000	454
D12980 *TBD*	see Notes	FF,L	H_2O	510	ND	556	pH 3	525	ND	556
D12981 *TBD*	see Notes	FF,L	H_2O	511	ND	556	pH 3	525	ND	556
F362	1182.54	FF,D,L	see Notes	476	32,000	519	MeOH/H^+	496	88,000	519
H22730	400.60	L	DMSO, EtOH	325	15,000	385	MeOH/H^+	366	20,000	453
L22460	see Notes	F,D,L	H_2O	384	ND	540	pH 3	329	ND	440
O6139	609.43	F,D,L	DMF, DMSO	489	26,000	526	pH 3	506	85,000	526
O6147	509.38	F,D,L	DMF, DMSO	480	24,000	521	pH 3	495	76,000	521
O6149	509.38	F,D,L	DMF, DMSO	480	26,000	516	pH 3	496	82,000	516
O12650	1086.25	FF,D,L	see Notes	485	26,000	526	MeOH/H^+	501	85,000	526
S22801	592.56	F,D	DMSO	<350		none		<350		non

TBD A product which is to be discontinued; only available as long as current stock remains.

Notes

1. Spectra are in aqueous buffers adjusted to >1 pH unit above and >1 pH unit below the pK_a.
2. Spectral data for this product represents the unreacted succinimidyl ester. The pK_a value and the spectral data for acidic solutions have been estimated based on the spectra of the parent carboxylic acid.
3. Examples of pH-dependent spectra for C3062 conjugated to peptides have been published

elsewhere (Cytometry 15, 148 (1994)).

4. Molecular weight is nominally as specified in the product name but may have a broad distribution.
5. ND = not determined.
6. Abs, Em and pK_a values listed for this dextran conjugate are those obtained for the free dye. Values for actual conjugates are typically very similar, with slight variations between different production lots.
7. These conjugates contain both pH-sensitive fluorescein (Abs = 495, Em = 520 nm) and pH-insensitive tetramethylrhodamine (Abs = 555 nm, Em = 575 nm) fluorophores.
8. The pK_a value and spectral parameters may vary between different lots of this product.
9. The pK_a values of lipophilic pH indicators may vary considerably depending on the electrostatic properties of membrane surfaces (Biochemistry 32, 10057 (1993); J Phys Chem 81, 1755 (1977); Biochim Biophys Acta 939, 289 (1988)). The pK_a values listed are for electrostatically neutral liposomes or micelles. Spectra are in MeOH containing a trace of HCl ($MeOH/H^+$) or a trace of KOH ($MeOH/OH^-$).
10. The pK_a of D12760 bound to phospholipid bilayer membranes is ~6.1.
11. Chloroform is the most generally useful solvent for preparing stock solutions of phospholipids (including sphingomyelins). Glycerophosphocholines are usually freely soluble in ethanol. Most other glycerophospholipids (phosphoethanolamines, phosphatidic acids and phosphoglycerols) are less soluble in ethanol, but solutions up to 1–2 mg/mL should be obtainable, using sonication to aid dispersion if necessary. Labeling of cells with fluorescent phospholipids can be enhanced by addition of cyclodextrins during incubation (J Biol Chem 274, 35359 (1999)).
12. C6826 and S22801 are converted to fluorescent products with spectra similar to C1270 after acetate hydrolysis.

* * * * *